Sleep Homeostasis in Juvenile Rats and in a Mouse Model of Depression

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1 Summary

Sleep, in both humans and rodents, is highly regulated and based on similar principles. In general, sleep is regulated by circadian and homeostatic processes. While the circadian process is responsible for the correct timing of sleep within 24 hours, the homeostatic process depends on prior sleep/wake history. In the electrocorticogram (ECoG), the homeostatic process is best reflected by slow wave activity (SWA, defined as ECoG power between 1 and 4.5 Hz) during non-rapid eye movement (NREM) sleep and increases as a function of prior wakefulness. It is thus maximal at sleep onset and steadily declines across a sleeping period. More recently, evidence has accumulated indicating that SWA in humans, as well as in rodents, provides a reliable marker for cortical plasticity. SWA is thought to reflect synaptic strength which increases during wakefulness and decreases back to its baseline level during subsequent sleep. The neuronal correlate of SWA is thought to be the degree of synchronicity of cortical neurons during NREM sleep. In particular, it has been shown that the more synchronous neurons fire, the higher the SWA.

The aim of the present thesis was to investigate sleep homeostasis in the rat by means of ECoG SWA during a period associated with increased cortical plasticity, such as during brain maturation [5.1 and 5.2], and during a period related to reduced cortical plasticity, such as in a recently developed mouse model of depression [5.3].

From human studies, it is known that cortical synaptic density follows an inverted U-shaped developmental trajectory, showing a strong increase during childhood followed by a decline during adolescence. The same also applies to the rat. A higher synaptic density means a better-connected neuronal network and results in higher synchronicity of cortical activity. Accordingly, SWA in humans was shown to follow a similar inverted U-shaped developmental trajectory matching the trajectory observed for synaptic density. As a consequence, SWA in humans can be used as a marker of cortical maturation. More recently, SWA was additionally proposed to play an active role in cortical maturation. This question was addressed in the first study presented in this thesis by assessing behavioural, structural and

electrophysiological markers of maturation [5.1]. SWA was manipulated by means of caffeine administration during a critical period of maturation. Caffeine is a known stimulant, which has been shown to reduce SWA in humans as well as in rats. Our results showed that in rats, SWA follows an inverted U-shaped developmental trajectory, similar to what is observed in humans. During pre-puberty, SWA continuously increased, reaching a peak SWA at about postnatal day 30 (P30). This was followed by a steady decline during puberty. Caffeine administration exerted short-term stimulating effects manifested by an increase in wakefulness and additionally altered the trajectory of SWA resulting in a delayed decline of SWA. Moreover, caffeine treatment resulted in a reduced increase in the explorative behavior across age when compared to the control group. Similarly, a structural marker of brain maturation, shown to decrease across age, showed higher values after caffeine administration indicating delayed maturation. Thus, caffeine consumption during this critical developmental period induced long-lasting effects on sleep and brain maturation.

As mentioned above, SWA accumulates during a waking period and decreases during a sleeping period. These increases and decreases of SWA across 24 hours are balanced in adulthood. The second study [5.2] investigated sleep homeostasis by assessing SWA across 24 hours in development (same data set as in 5.1) and in more mature rats. We investigated the time course of SWA across 24 hours for specific postnatal days that are predominately associated with synaptogenesis (<P30) or pruning (>P30), respectively. We observed a gain of SWA across 24 hours during the period associated with synaptogenesis. On the contrary, a loss of SWA was found during the period associated with pruning. As expected, more mature rats did not show any net changes in SWA across 24 hours. Thus, SWA during maturation does not only reflect sleep homeostasis but also maturational processes.

Based on the observation that sleep deprivation leads to a reduction of depressive symptoms, it has been suggested that SWA accumulates at a slower rate in people suffering from depression. Accordingly, in depressed patients, SWA only reaches normal levels after prolonged waking. In the third study [5.3], we investigated sleep homeostasis in mice after they underwent a chronic stress protocol known to

induce depressive-relevant behaviour. The ECoG was recorded for two consecutive days (day 1: baseline; day 2: sleep deprivation and recovery). As expected, sleep deprivation led to an increase in SWA. However, mice that had previously underwent a chronic stress protocol showed a blunted increase in SWA when compared to control mice. Thus, during prolonged wakefulness, SWA accumulated at slower rate in chronically stressed mice. Based on the close relationship between SWA and synaptic plasticity, this might reflect impaired plasticity during wakefulness in chronically stressed mice.

In summary, these studies confirm the close relationship between SWA and synaptic plasticity in the course of cortical maturation as well as in a mouse model of depression. SWA can be used to mirror cortical maturation and to uncover alterations of cortical maturation. In addition, SWA measurements in a mouse model of depression provide further evidence that impaired plasticity processes constitute one of the mechanisms underlying depressive behaviour.

2 Zusammenfassung

Schlaf unterliegt einer ausgeprägten Regulation, welche sowohl im Mensch wie auch im Nagetier auf ähnlichen Prinzipien beruht. Generell wird der Schlaf von einem zirkadianen und einem homöostatischen Prozess reguliert. Während der zirkadiane Prozess für den korrekten Zeitpunkt der Schlafperiode innerhalb von 24 Stunden zuständig ist, hängt der homöostatische Prozess von vorherigen Schlaf-Wach Episoden ab. Der homöostatische Prozess widerspiegelt sich in den mittels Elektrokortikogramm (EKoG) gemessenen langsamen Wellen (Slow Wave Activity, SWA, definiert als die EKoG-Aktivität zwischen 1 und 4.5 Hz) des Non-Rapid Eye Movement (NREM) Schlafs. SWA steigt dabei in Abhängigkeit von der vorherigen Wachdauer an. Demzufolge erreicht SWA Maximalwerte zu Beginn einer Schlafperiode und nimmt während des Schlafs kontinuierlich ab. Ergebnisse aktueller Studien unterstützen die Hypothese, dass SWA im Menschen sowie im Nagetier ein zuverlässiger Indikator für kortikale Plastizität ist. Es wird dabei angenommen, dass SWA die synaptische Stärke widerspiegelt, die sich während einer Wachdauer kontinuierlich aufbaut und während des anschliessenden Schlafs auf das Ausgangs-Niveau reduziert wird. Durch die Erforschung des neuronalen Korrelats der SWA wurde ein enger Zusammenhang zwischen der Synchronizität der neuronalen Aktivität während des NREM Schlafs und der SWA gefunden: je synchroner die neuronale Aktivität während dem NREM Schlaf ist, desto mehr SWA kann gemessen werden.

Im Mittelpunkt der vorliegenden Dissertation stand die Untersuchung der Schlafhomöostase mittels EKoG SWA während einer Periode erhöhter kortikaler Plastizität sowie während einer Periode erniedrigter Plastizität. Die Periode der erhöhten Plastizität wurde dabei während der Gehirnmaturierung in Ratten gemessen [5.1 und 5.2], wobei ein kürzlich entwickeltes Maus-Modell für Depression es erlaubte, die Schlafhomöostase während einer Periode, die mit einer erniedrigter kortikalen Plastizität assoziiert wurde, zu erforschen [5.3].

Studien im Menschen haben gezeigt, dass die kortikale synaptische Dichte während der Entwicklung eine invertierte U-Form beschreibt. Das heisst, die synaptische Dichte steigt während der Kindheit an, gefolgt von einem Abfall während der Adoleszenz. Ein ähnlicher Verlauf der synaptischen Dichte konnte auch in der Ratte gezeigt werden. Eine erhöhte Synapsendichte führt zu einem stärker verknüpften neuronalen Netzwerk, was wiederum eine höhere Synchronizität der kortikalen Aktivität zur Folge hat. Entsprechend konnte im Menschen gezeigt werden, dass SWA einen ähnlichen Entwicklungsverlauf zeigt wie die Synapsendichte. Demzufolge stellt SWA im Menschen ein Indikator für kortikale Maturierung dar. Kürzlich wurde vorgeschlagen, dass SWA sogar eine aktive Rolle in der kortikalen Maturierung spielen könnte. Diese Hypothese wurde in der ersten Studie [5.1] mittels struktureller, verhaltenspezifischer und elektrophysiologischer Parameter untersucht. In dieser Studie wurde SWA während einer kritischen Reifungsperiode mittels Koffeinadministration manipuliert. Neben seiner stimulierenden Wirkung, reduziert Koffein im Menschen sowie in der Ratte SWA. Aus der Analyse der erhobenen Daten ging hervor, dass SWA in der Ratte einen ähnlichen Entwicklungsverlauf zeigt, wie bereits aus Humanstudien bekannt. Während der vorpubertären Periode stieg SWA kontinuierlich an, erreichte Maximalwerte am ~postnatalen Tag 30 (P30), die von einer stetigen Abnahme während der pubertären Periode gefolgt wurden. Die Verabreichung von Koffein hatte einen kurzfristigen stimulierenden Effekt, der durch eine Verlängerung der Wachdauer gekennzeichnet war. Darüber hinaus führte die Koffeingabe langfristig zu einem deutlich veränderten Entwicklungsverlauf der SWA. Der bei den Kontrolltieren gemessene Abfall der SWA war bei den Tieren der Koffeingruppe verzögert. Das explorative Verhalten, welches sich in der Kontrollgruppe mit zunehmendem Alter erhöhte, zeigte eine verminderte Zunahme in Ratten, die Koffein verabreicht bekommen hatten. Weiter zeigte ein struktureller Marker der Gehirnmaturierung, der mit zunehmendem Alter abnahm, signifikant erhöhte Werte in der Koffeingruppe, verglichen zur Kontrollgruppe. Aufgrund der Resultate waren also alle Marker der Gehirnmaturierung nach Koffeinabgabe verzögert. Demzufolge hat der Koffeinkonsum während einer kritischen Maturierungsperiode in der Ratte lang anhaltende Auswirkungen auf den Schlaf und die Gehirnmaturierung.

Wie bereits beschrieben, steigt SWA während der Wachphase an und fällt während der Schlafperiode ab. Im Erwachsenenalter ist die Zu- und Abnahme der SWA über die Zeit in Balance. In der zweiten Studie [5.2] wurde die Schlafhomöostase in der Ratte mittels SWA über 24 Stunden während der Entwicklung (gleicher Datensatz wie für [5.1]), wie auch in etwas älteren Ratten untersucht. Wir untersuchten den SWA Verlauf innerhalb von 24 Stunden an verschiedenen postnatalen Tagen, die entweder mit einem Übermass an Synaptogenese (<P30) oder Pruning (>P30) assoziiert wurden. Dabei fanden wir eine Zunahme von SWA während der Tage, die mit erhöhter Synaptogenese assoziiert wurden, wobei an Tagen mit erhöhtem Pruning eine Abnahme von SWA gefunden wurde. Die älteren Ratten zeigten keine Nettoveränderung von SWA über 24 Stunden. Dieses Resultat weist darauf hin, dass SWA während der Entwicklung nicht nur die Schlafhomöostase, sondern zusätzliche Maturierungsprozesse widerspiegelt.

Vor dem Hintergrund, dass eine Schlafdeprivation zu einer Reduktion von depressiven Symptomen führt, wurde vermutet dass SWA während der Wachheit in depressiven Patienten langsamer akkumuliert und erst nach einer verlängerten Wachdauer ein normales Niveau erreicht. In Studie 3 [5.3] untersuchten wir demzufolge die Schlafhomöostase bei Mäusen, bei denen mittels chronischer Stressexposition ein depressiver Phänotyp induziert wurde. Dabei wurde der Schlaf während zwei aufeinanderfolgenden Tagen (Tag 1: Baseline, Tag 2: Schlafdeprivation und Erholungsphase) gemessen. Wie erwartet, stieg SWA nach der Schlafdeprivation signifikant an. Dieser Anstieg war jedoch bei den Mäusen, die zuvor das Stress-Protokoll durchliefen, deutlich reduziert. Aufgrund der engen Beziehung zwischen SWA und synaptischer Plastizität lässt sich vermuten, dass Mäuse, die einem Stress-Protokoll unterzogen wurden, eine beeinträchtigte neuronale Plastizität aufweisen.

Insgesamt bestätigen diese Studien eine enge Beziehung zwischen SWA und synaptischer Plastizität während der Entwicklung, wie auch in einem kürzlich entwickelten Maus-Modell für Depression. SWA ist ein valider Indikator der kortikalen Maturierung und kann darüber hinaus Abweichungen der kortikalen Maturierung

abbilden. Die Ergebnisse der veränderten SWA-Homöostase im Depressions-Mausmodell deuten darauf hin, dass die der Depression zugrunde liegenden krankhaften Veränderungen mit verminderter Plastizität in Verbindung gebracht werden können.

3 Statement of the candidate's contribution to this thesis

All of the data analysis, figure production and writing in this thesis was done by Nadja Olini. The work for the review and the 3 paper sections of this thesis was performed primarily by Nadja Olini (with normal supervisorial input from PhD Reto Huber), with her contribution to each project as described below.

[1] **Sleep in all stages of human development.**
The literature research as well as the writing was performed by Nadja Olini.

[2] **The effects of caffeine on sleep and maturational markers in the rat.**
The experiments were planned, carried out, analysed and written by Nadja Olini.

[3] **Diurnal changes in EEG sleep slow wave activity during development in rats.**
The experiments were planned, carried out, analysed and written by Nadja Olini.

[4] **Sleep homeostasis in a mouse model for depression.**
The study design, experimental work and analyses were carried out by Iru Rothfuchs and Nadja Olini in collaboration with PD Christopher Pryce. The writing combined with additional data analyses was performed by Nadja Olini.

Nadja Olini (candidate) Date

4 Introduction

Many different functions have been suggested for sleep. In general, the proposed hypotheses can be divided into three main groups: First, sleep serves to reduce energy expenditure; Second, sleep is needed for the restoration of key cellular component and for macromolecule biosynthesis and third, sleep facilitates learning and memory by means of plasticity processes (Mignot, 2008). In recent years, a body of literature emerged emphasizing the importance of sleep for cortical plasticity. In line with the latter, the present thesis investigates sleep in relation to cortical plasticity.

Although the function of sleep is still a matter of debate, we have good knowledge about how sleep manifests itself. Sleep as a state can be behaviourally defined by 6 criteria: (1) decreased behavioral activity, (2) site preference, (3) specific body posture (4) reversibility between sleep and wake (unlike coma), (5) increased arousal threshold and (6) homeostatic regulation (rebound after sleep deprivation) (Zeplin, 2000, Campbell and Tobler, 1984, Tobler, 2005). Based on its behavioral definition, this recurring state may appear to be a homogeneous state of reduced mobility and responsiveness to external stimuli. However, when assessed by means of polygraphic measurements, sleep appears to be a heterogeneous and highly regulated state.

The regulation of sleep, for example, ensures that sleep does not occur randomly across 24 hours. This is however not the case in newborns. Thus, sleep regulation at birth is immature and undergoes dramatic changes during early human development. Even after the establishment of the processes underlying sleep regulation, these processes keep changing throughout lifetime. These age-related changes in the regulation of sleep constitute a hallmark of sleep at different ages and have contributed to a better understanding of the function of sleep. The following section introduces sleep at all stages of human development by providing an overview of the main changes of the characteristics of sleep across development and of how they are related to the regulation of sleep.

4.1 REVIEW: Sleep in all stages of human development

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SUMMARY

Sleep across life shows prominent changes: Sleep duration and architecture change mainly during infancy and early childhood, the regulation of sleep undergoes considerable changes during adolescence and adulthood. In this review we provide an overview of specific hallmarks of sleep during different developmental stages. Most of the knowledge is based on electrophysiological measurements starting at birth when the electroencephalographic (EEG) traces display a discontinuous pattern, which is followed by the transition from neonatal sleep to infantile sleep. Non-rapid eye movement (NREM) and rapid eye movement (REM) sleep originate during this period. Both sleep stages undergo further changes during childhood, adolescence and adulthood.

The regulation of sleep depends on two main processes, a homeostatic process reflecting sleep pressure, which can be assessed by slow wave activity (EEG power, 1-4.5 Hz) during NREM sleep and a circadian process responsible for the correct timing of sleep within 24 hours. Both systems evolve during the first years of life and show maturational changes, most prominently during childhood and adolescence. During childhood and adolescence the circadian process steadily delays and the homeostatic process undergoes marked changes in its dynamics and characteristic. During early adulthood both processes remain stable. Typical modifications of sleep in elderly are decreased sleep quality and quantity. Moreover, EEG spectral power during NREM sleep gradually decreases after the age of 30, which was associated with changes in brain structure and related to a decline in memory performance with increasing age.

Sleep at birth is markedly different from sleep later in childhood and adulthood. Thus, our chapter is subdivided into three main developmental windows, infancy, childhood/adolescence, and adulthood. Since sleep is changing during development in a multidimensional way we are looking at changes in sleep duration/architecture as well as changes in the major electrophysiological characteristics. The major tool to obtain these measures is electroencephalography. Hence a major focus will be to highlight the changes in the electroencephalogram (EEG).

SLEEP DURING INFANCY (0 TO 1 YEAR)

Sleep stages at birth

First EEG recordings are possible after birth. Recordings of premature infants provide a means to gain insights into brain activity before birth. When looking at the EEG in premature infants the general picture changes significantly until full term is reached. The EEG shows a discontinuous pattern, consisting of periods of electrical activity, called bursts, separated by periods of inactivity, called interburst intervals. When the premature neonate reaches full term, the duration of these bursts become longer, whereas the duration of the interburst interval decreases. Eventually, shortly after term, the EEG becomes continuous (Hayakawa et al., 2001).

During all development stages EEG is combined with other polygraphic parameters like electromyography (EMG) measuring the muscle tone and electrooculography (EOG) tracking eye movements to distinguish the different sleep stages. To assess sleep stages in infants additional polygraphic parameters like the electrocardiogram (ECG) and respiration measurements together with behavioral observation via video recording are often used (Anders et al., 1971). After approximately 2 months of age sleep stages in infants can be termed REM/NREM sleep as done in adults. However, before this age, terminology for sleep stages is different since the EEG pattern and the organization of sleep looks different compared to adults (Fig. 4.1.1). What develops into REM sleep is initially termed active sleep (AS) and NREM sleep at this early age is classified as quiet sleep (QS).

Moreover, an additional sleep stage, not present at older age, called indeterminate sleep (IS) is used (Iber et al., 2007).

The EEG during AS is characterized by continuous mixed theta (4-8 Hz) activity with some delta (1-4.5 Hz), alpha (8-13 Hz) and beta (12-30 Hz) activity of 40-80 μV amplitude. Meanwhile, the EOG shows slow and rapid bursts of eye movements including isolated eye movements. The muscle tone during AS is of low amplitude superimposed with twitches and phasic jerky movements (e.g. sucking motions, smiling) while the respiration is irregular in both, rate and depth. In contrast, during QS the EEG displays a tracé alternant consisting of bursts of high amplitude slow waves (0.5-3 Hz) separated by low voltage mixed frequency EEG (Ellingson and Peters, 1980). Eye movements are mostly not present although infrequently eye movements can be observed. Moreover, the ECG and respiration are both predominantly regular during QS in contrast to AS. The EMG at that early stage displays a similar pattern during QS and AS and thus does not provide a valuable measure to distinguish between AS and QS (Mirmiran et al., 2003). When sleep cannot be allocated to AS nor QS it is termed IS. IS is therefore frequently used during transitions between QS and AS and its occurrence fades during the first few months of life.

Thus far, all three precursor sleep stages present at birth have been introduced. The following few months of life are characterized by the development of these precursor sleep stages into more mature sleep stages, which can be distinguished throughout lifetime. Therefore the next section will focus on the transition from neonatal to infantile sleep.

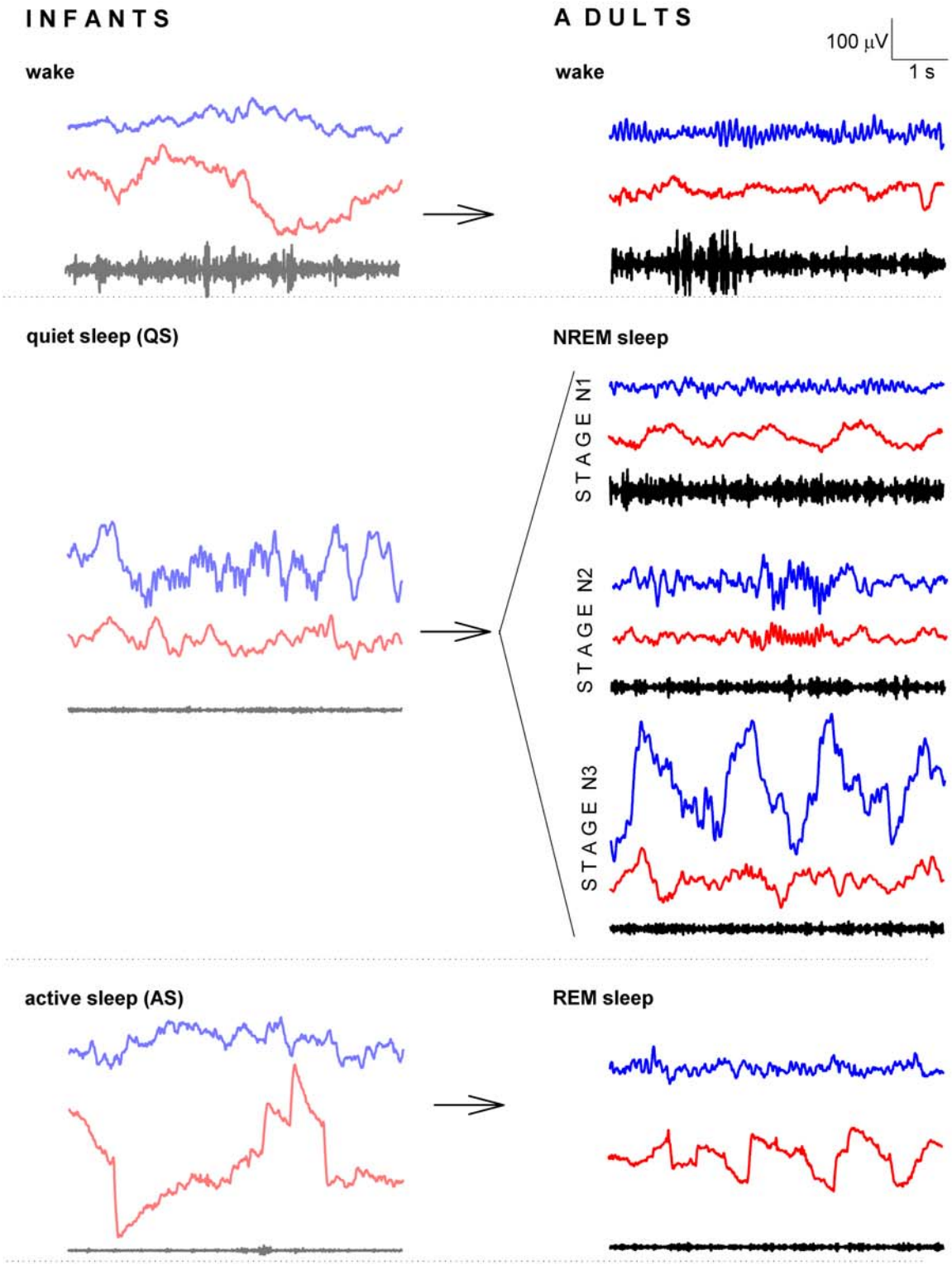
INFANTS
ADULTS
100 µV
1 s
wake
wake
quiet sleep (QS)
NREM sleep
STAGE N1
STAGE N2
STAGE N3
active sleep (AS)
REM sleep

Figure 4.1.1: Electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG) in infants and adults. 6-s sample EEG (in blue), EOG (in red) and EMG (in black) traces are illustrated for all vigilance states for a 2 months old infant and a 27 years old adult. Infants precursor sleep stages are subdivided in quiet sleep (QS) and active sleep (AS) which develop into NREM and REM sleep.

From neonatal to infantile sleep

The transition between neonatal and infantile sleep occurs gradually over a period of many weeks and is usually completed at 3-6 months. Neonatal sleep is characterized by sleep onset through AS which diminishes across the first 3 months and gradually disappears when the newborn enters infantile sleep (Anders and Keener, 1985). Another hallmark of neonatal sleep is the tracé alternant during QS characterized by bursts of theta and delta activity reaching amplitudes as high as 300 µV followed by periods of faster mixed activity with amplitudes between 25 to 50 µV. At term the bursts last from 2 to 4 s and alternate with periods of lower amplitude but similar duration. With two weeks of age the tracé alternant starts fading by shortening the interburst intervals and gradually disappears over the first two months (Ellingson and Peters, 1980).

At the time when QS evolves into NREM sleep, in most infants beyond the age of 4-6 months a subdivision becomes standard: NREM sleep is subdivided into stages N1, N2 and N3. Stages N2 and N3 form so called slow wave sleep (SWS). This subdivision becomes possible because at the transition between neonatal and infantile sleep important EEG hallmarks, sleep spindles and K-complexes, typically occurring during N2 sleep, emerge. Both of these hallmarks are suspected to be important for normal brain maturation as they are thought to play a role in the development of the cerebral cortex as well as in processes of memory and learning (Dan and Boyd, 2006, Timofeev et al., 1996). Sleep spindles are produced from rhythmic spike bursts in GABAergic neurons that arise from synchronized activities in neuronal networks that link the thalamus to the cortex and occur at 11-15 Hz. This activity develops during the first 3 months of life. The appearance of sleep spindles in the EEG is associated with a more matured interplay between the thalamus and the cortex. Initially they occur asynchronously and can best be seen over the central

regions of the head. During subsequent months the occurrence of sleep spindles become more synchronous and an increasing proportion of spindles appear synchronous and symmetrical between hemispheres, i.e. the percentage of synchronous spindles rises from about 50% at the age of 6 months to 70% at 1 year (Ellingson and Peters, 1980). The characteristics of sleep spindle activity changes with maturation in terms of frequency, amplitude, length, and density and may therefore serve as an index of neural maturation (Jenni et al., 2004, Scholle et al., 2007, Louis et al., 1992). Sleep spindles show an increase in frequency and amplitude during the first 6 months. Moreover, spindle density and length follow an inverted U-shaped trajectory: they increase during the first 3 to 6 months of life followed by a decline, reaching minimal values at 2 years of age. K-complexes appear around 5 months of age, are usually present by 6 months (Metcalf et al., 1971) and often precede spindle sequences. The underlying cellular mechanism of K-complexes is based on a slow (<1 Hz) oscillation of cortical neurons associated with fluctuations of the membrane potential between a depolarized and a hyperpolarized level (Amzica and Steriade, 1997).

Changes in sleep duration and sleep architecture

While the transition of newborn to infantile sleep is not easily observable for parents because the changes are best reflected in polygraphic measurements, parents most certainly experience the prominent changes their children go thorough in terms of the amount of sleep and its distribution across a day. Average total sleep time (TST) decreases during the first year of life from approximately 16 hours to 13 hours (Fig. 4.1.2). In addition, a huge variability in TST is apparent especially during the first few months after birth which then decreases over time (Iglowstein et al., 2003). For example, sleep duration in neonates varies between 9 and 19 hours, which decreases to 11 to 16 hours in 1 year old infants. During the same time the amount of AS massively decreases from 50% at birth to approximately 30% of TST after the first year of live, approaching adult values of 25-30% (Roffwarg et al., 1966). The decline of AS or REM sleep (>2 months), respectively, can also be seen in other mammals such as rats, which undergo most of their brain development postnatally. The guinea pig, in contrast, has most of its CNS development before birth and

exhibits adult REM sleep levels at birth (Jouvet-Mounier et al., 1970). Based on these species differences, the amount of AS/REM sleep at birth was proposed to be a marker of the developmental status at birth. Hence, AS or REM sleep, both characterized by an EEG pattern similar to the waking EEG, were already in 1966 thought to play an important role for brain maturation during a developmental period when waking is limited (Roffwarg et al., 1966). While during the first year of life AS or REM sleep markedly decline in their duration and also when expressed as a percentage of TST, the amount of QS increases (Hoppenbrouwers, 1987). Similar to AS, IS steadily declines over the first few months and can no longer be identified at 6 months (Iber et al., 2007).

The different sleep stages show a typical sequence. Such a sequence is forming a sleep cycle, which is characterized by the alternation between NREM and REM sleep. The occurrence of these cycles across sleep is also termed ultradian sleep rhythm. Already at birth an ultradian sleep rhythm is present, however, it differs from sleep rhythms later in life. A major change is the duration of a sleep cycle: During infancy the ultradian rhythm lasts about 50 minutes and gradually increases with age until adult levels of 90 to 110 minutes are reached around school age. Moreover, while initially AS and QS are of similar duration across a sleep cycle, with increasing age REM sleep predominates at later sleep cycles and deep NREM sleep dominates sleep cycle during the earlier part of sleep.

Towards the end of the first year infants show a clear predominance of sleep during the night and one or two daytime naps. At that age all sleep stages can be distinguished based on EEG, EOG and EMG. NREM sleep is now characterized by low frequency (1-4.5 Hz), high voltage EEG activity, low muscle tone and the absence of eye movements and can be subdivided in 3 stages. Stage N1 NREM sleep occurs at sleep-wake transitions, stage N2 is characterized by sleep spindles and K-complexes and stage N3 NREM sleep exhibits the highest amplitude waves in the low frequency range. During the same age span not only the typical hallmarks of sleep mature but also the regulation of sleep undergoes dramatic changes.

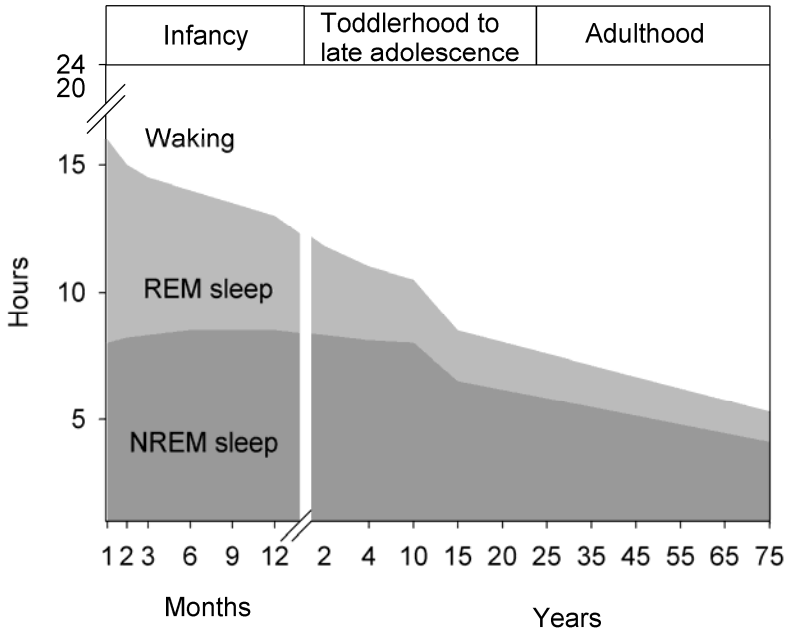

Figure 4.1.2: Changes in vigilance states distribution across life. Note that sleep architecture dramatically changes across life with a prominent reduction of both, total sleep time and REM sleep during the first years of life followed by minor changes during adolescence (12-18 years) (redrawn from Roffwarg et al., 1966).

Sleep regulation during infancy

One of the biggest changes a newborn experiences is the division of 24 hours into light-dark cycles. Although babies show in utero periods of activity and inactivity this rhythmic behaviour does not correlate with the light-dark cycle. Therefore it is not surprising that newborns at birth show hardly any circadian preference for sleep. This circadian organization evolves subsequently and light exposure is closely related to its development.

Sleep regulation depends on two intrinsic processes and can be best described by the two-process model of Borbély (Borbely, 1982). A sleep-wake dependent homeostatic process S reflects the increase of sleep pressure during waking and a recovery process with a dissipation of sleep pressure during subsequent sleep. The homeostatic process interacts with a sleep-wake independent process termed circadian process C which is responsible for the correct timing of sleep within 24

hours. The anatomical structure responsible for the circadian rhythm generation is located in the suprachiasmatic nucleus of the hypothalamus (SCN). Although SCN neurogenesis has been detected in humans as early as 18 weeks conceptional age and its underlying neurons show already rhythmicity at birth (Reppert et al., 1988b), the circadian rhythm at this age is not fully established (Swaab et al., 1990), probably due to underdeveloped input/output pathways. Therefore, sleep at this age is arrhythmic and occurs as easily during the daytime hours as during the night. This is in line with the absence of a circadian modulation of physiological parameters like hormone secretion (e.g. melatonin, serotonin) or body temperature (Rivkees and Hao, 2000). During the transition from neonatal to infantile sleep circadian rhythms (e.g. melatonin or temperature) become apparent and are accompanied by the ability to stay awake for longer duration with more consolidated sleep episodes during the night (Kleitman and Engelmann, 1953, Parmelee, 1961). Thus, at this age, night time sleep increases, while the duration of day time sleep decreases.

There is a considerable variability in when circadian rhythms appear after birth, ranging from the first to several months after birth. This huge variability may be due to several factors including feeding (e.g. scheduled vs. on demand) and environmental lighting (e.g. regular vs. irregular light-dark cycle). A carefully performed study with controlled environmental and feeding conditions revealed a clear circadian rhythm in body temperature within the first week of life. Stable circadian rhythms of sleep-wake and melatonin emerged by ~6 weeks of age (McGraw et al., 1999). The importance of the lighting condition during these early weeks of life was also shown in a study showing beneficial effects of cycled lighting versus dim lighting in a neonatal care study (Guyer et al., 2012). Taken together, the circadian system undergoes massive changes within the first months after birth. At 6 months of age typically the circadian timing becomes mature and remains relatively stable during childhood.

Also the homeostatic process regulating sleep pressure undergoes developmental changes. It is generally assumed that in infants sleep pressure accumulates faster during waking and dissipates faster during sleep compared to adults. This observation would explain the inability of neonates to maintain consolidated periods of waking or sleep. Accordingly, it has been shown that short periods of sleep deprivation resulting in negligible effects in adults, lead in infants to a

rapid increase in sleep pressure and produce a compensatory increase in sleep time and/or intensity during recovery (Anders and Roffwarg, 1973, Thomas et al., 1996). In adults, sleep pressure is best reflected by sleep slow-wave activity (SWA, EEG spectral power between 0.5 and 4.5 Hz frequency range) during NREM sleep. SWA increases as a function of prior waking and progressively dissipates during subsequent sleep (Borbely and Achermann, 2005). While in adults sleep deprivation solely leads to intensified sleep during recovery sleep, reflected by an initial increase in SWA, followed by a steady decline in the course of subsequent sleep, the same homeostatic response in infancy can not be found. Instead, infants as well as young rats, initially respond to sleep deprivation with an increase in sleep duration and only at a later stage with intensified sleep, characterized by more SWA (Anders and Roffwarg, 1973, Frank et al., 1998, Thomas et al., 1996). For example, sleep deprivation in a pre-pubertal rat at 12 days of age leads to extended sleep duration during recovery (Frank et al., 1998). However, only 12 days later, sleep deprivation results in an increase in SWA, similar to adult rats, with no extension of sleep duration. A similar picture was found in neonates. Here, selective or total sleep deprivation led to a compensatory increase in NREM sleep duration only (Anders and Roffwarg, 1973, Thomas et al., 1996). The exact age at which sleep deprivation in neonates or infants leads to a compensatory response of SWA is not known. However, another important feature of the homeostatic process, the decline of SWA across the night, was already evident during the second postnatal months (Bes et al., 1991). Other studies showed during this time window an alternating pattern of SWA with high values in every second NREM sleep episode (Jenni et al., 2004). In the study by Jenni et al theta activity exhibited a declining trend across consecutive sleep episodes. Therefore, while in adults sleep pressure is reflected by SWA, there is evidence that theta activity may be a marker for sleep pressure in infants.

As shown in the previous paragraphs, the homeostatic and the circadian process develop during infancy. However, sleep regulation depends on both the homeostatic and the circadian processes making it difficult to separate the influence of the timing of the sleep-wake schedule from that of the circadian pacemaker. One way to disentangle the homeostatic from the circadian process is the so called forced desynchrony protocol. In this protocol the separation is achieved under conditions in which subjects are scheduled to rest-activity cycles that are outside the range of

entrainment of the circadian pacemaker, which continuous to oscillate at its intrinsic period of ~24.2 h. These protocols help to scheduled sleep and wake episodes at virtually all circadian phases making it possible to disentangle the two processes. Thus far, several studies applied the forced desynchrony protocol in both young and old people, however no such study was performed during infancy making it difficult to relate the observed changes to the homeostatic or the circadian process.

SLEEP FROM TODDLERHOOD TO LATE ADOLESCENCE (1 TO 25 YEARS)

Changes in sleep duration and sleep architecture

As previously introduced, the first year of life is characterized by the maturation of sleep stages as well as the development of sleep regulation per se. However, once the basic principles of sleep are established sleep characteristics and sleep regulation continue to change (Fig. 4.1.2). In toddlers (1 to 3 years) and preschoolers (3-5 years), changes in sleep-wake characteristics occur more slowly than during the preceding months of life. Daily sleep duration further decreases mostly due to a gradual sleep onset shift from ~8 PM to 9, 9.30 PM, while the awakening time across this age span remains relatively constant at 7 AM. Meanwhile, napping decreases from at least one nap at 2 years to the absence of naps in 4 to 5 years old children. The reduction of day time naps is accompanied by an increase in sleep efficiency, defined as the percentage of time spent asleep when lying in bed, especially during the second year and remains constant thereafter until age 5 (Acebo et al., 2005, Meltzer and Mindell, 2006). During pre-school age REM sleep percentage gradually declines and approaches adult levels of 20%-25% (Roffwarg et al., 1966). Moreover, the duration of NREM/REM sleep cycles steadily increases from 40 min at 2 years to 60 min at 5 years of age, approaching adult values of 90 to 110 min. At this age children have 7 to 10 cycles during each nocturnal sleep period (Kahn et al., 1996). A commonly observed phenomenon in toddlers and pre-schoolers are night wakings assumed to reflect a remnant of the ultradian rhythm of sleep cycles (Fazzi et al., 2006). Later on, in school-age children (6-10 years) TST further declines to about 10 hours a night. This duration is still markedly above adult levels.

During adolescence (12-18 years) time spent in bed and TST further decrease across age. More specifically, pre-pubertal children (<12 years) sleep ~10 hours a night (Russo et al., 2007), midadolescents 8.5 hours, and older adolescents about 7 hours a night (Roffwarg et al., 1966). This reduction in TST during adolescence was initially thought to result from a reduced sleep need across adolescence. However, subsequent studies investigating TST between school nights and nonschool nights across adolescence found in older adolescents longer TST during weekends compared to school days (Ohayon et al., 2004). This longer sleep time at weekends may reflect recovery from accumulated sleep deprivation during the week in adolescents, probably due to the control of bedtimes and wake times by environmental factors like school work in the evening and waking up by the alarm in the morning during the week (Carskadon et al., 2004). In line with this, older adolescents reported more daytime sleepiness compared to younger adolescents (Sadeh et al., 2000). Although environmental factors such as school demands are believed to be partly responsible for the reduction of TST across adolescence, more recently, changes in biological processes that regulate sleep and wakefulness are also thought to impact sleep patterns during this developmental period. In addition to a gradual reduction in TST during adolescence, both the total amount of SWS per night, as well as the percentage of time spent in SWS during a nocturnal sleep episode significantly decrease between ~10 and ~17 years of age. During this developmental period, the percentages of REM and NREM sleep reach adult levels with longer REM sleep episodes in the course of the night (Kahn et al., 1996).

Changes in EEG-Spectral Power

In addition to the changes in sleep architecture across childhood and adolescence longitudinal studies revealed significant age-dependent changes in EEG spectral power values. During the initial years of life EEG power increases in particular in the slow wave frequency range during NREM sleep (Kurth et al., 2010). Maximal power values in the faster frequency bands like theta, alpha and beta activity are reached at 2 to 5 years of age. Power in these frequency bands steadily decrease thereafter. Power values in the SWA frequency range reach maximal values between 8 and 11 years. Similar to power in the faster frequencies during

NREM sleep, EEG power during wakefulness decreases in most frequencies after 6 years of age (Gasser et al., 1988). However, the most prominent reductions in power were found later during adolescence, in particular in SWA and theta activity during NREM sleep, showing a decline of about 60%. The steep decline is followed by an attenuated declining rate in both frequency bands at the age of 17 years (Feinberg and Campbell, 2010).

The observation, that absolute spectral power during all vigilance states decreases during adolescence suggest that, in general, neuronal EEG-generating mechanism change across adolescence. Already in 1977 Feinberg found that EEG power follows a similar trajectory as synaptic density (Feinberg et al., 1977). At birth synaptic density or a related measures gray-matter density (Fig. 4.1.3) (Paus et al., 2008) is low, increases steeply in the first years of life, reaches a maximum during childhood, and then declines across adolescence before it stabilizes during adulthood. Thus, in the course of this process of synapse elimination, synaptic density at age 20 reaches about half of that at 10 years of age (Huttenlocher, 1979). Campbell and Feinberg postulated that this prominent reduction in synaptic density, also called synaptic pruning, is reflected in the decrease of SWA (Feinberg and Campbell, 2010). While early life is characterized by an overproduction of synaptic connections adolescence is the age span when neuronal circuits get optimised by removing or pruning unnecessary connections.

But how might such a relationship between SWA and changes in connectivity be explained? SWA mainly reflects the signal amplitude in the slow wave frequency range. Recent evidence shows that a higher synchronicity of neuronal firing leads to more SWA (Vyazovskiy et al., 2009). A higher synchronicity is closely related to a better connected network (Whitlock et al., 2006). Therefore, the overproduction of neuronal connections during early life leading to higher synaptic density is thought to result in increased synchronicity, which in turn leads to an increase in SWA. During subsequent years, synaptic connections are lost, resulting in a decrease of synaptic density during puberty and adolescence. This decrease of synaptic density is related to a reduction of neuronal synchronicity, which then leads to a reduction of SWA. Hence, SWA and synaptic density follow a similar inverted U-shaped trajectory during childhood and adolescence. Interestingly, the loss of SWA does not occur simultaneously across all cortical regions but follows a posterior to anterior time

course. More specifically, maximal SWA in 2-5 years old children is located over the occipital cortex, shifts then over central regions, and finally reaches frontal regions in 11-14 years old adolescents (Fig. 4.1.3) (Kurth et al., 2010). MRI studies reported, that this posterior-anterior shift of SWA is accompanied by a similar developmental trajectory of gray matter volume which also shifts from posterior to anterior regions (Giedd, 2004). Taken together, SWA shows prominent age related changes, which might be explained by their relationship to synchronicity. Thus, SWA may serve as a marker for cortical maturational processes.

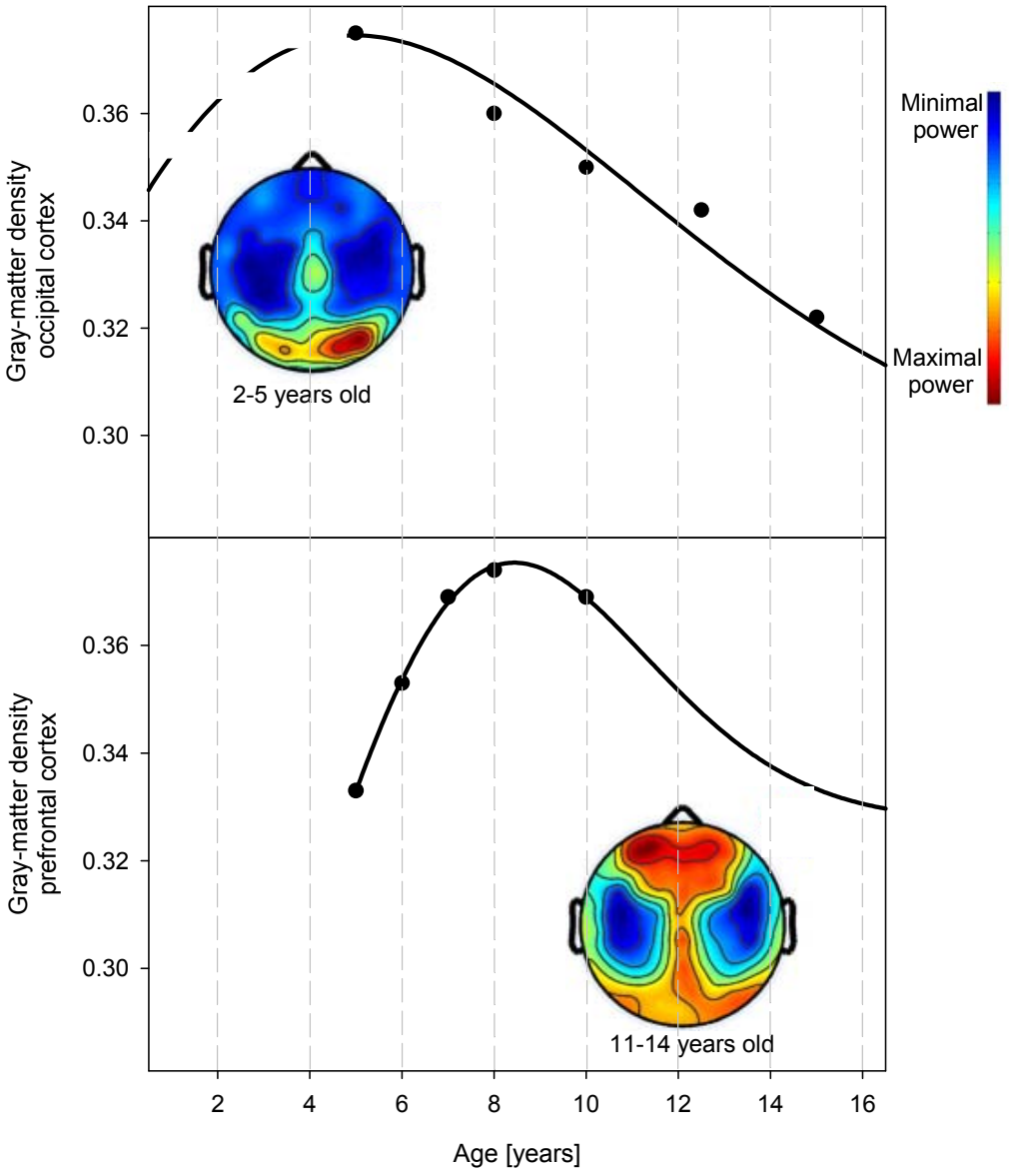

Figure 4.1.3: The topographic-specific relationship between gray-matter density and SWA across childhood and puberty. Gray-matter density follows an inverted U-shaped trajectory which peaks at different ages in the occipital and prefrontal cortex. Maximal power in the SWA frequency range in a specific region is reached approximately at the same age when gray-matter density peaks (redrawn from Kurth et al., 2010, Paus et al., 2008).

Circadian and homeostatic changes of sleep

While with 6 months of age the circadian system has developed and remains relatively stable during childhood, it again undergoes developmental changes during adolescence. Although the timing of sleep in adolescents has long been known to delay with increasing age, this observation has been attributed to external factors. However, Carskadon and colleagues first demonstrated that delayed bedtimes in adolescent not only derive from environmental factors but originate from a change in the circadian timing system (Carskadon et al., 1993). They found a correlation between delayed sleep phase and self-assessed pubertal development, even when grade level in school was held constant. Thus, under the assumption that teenagers attending the same grade in school are exposed to similar social environment, this evidence suggests that a biological component drives the adolescent sleep patterns. Moreover, the developmental timing of this phase delay parallels pubertal development. More specifically, girls show an earlier pubertal onset than boys and similarly it was found that also the phase delay was one year earlier in girls than in boys (Fig. 4.1.4). Subsequent studies in 16 countries on 6 continents, in cultures ranging from pre-industrial to modern found this gradual delay in the circadian system during puberty or adolescence (Carskadon, 2008). In addition, also other mammalian species showed such a phase delay during maturation (Golub et al., 2002, Tate et al., 2002). The underlying mechanism of this phase delay is not entirely understood but may include a phase delay of the circadian rhythm via increased evening light sensitivity leading to a delayed onset of melatonin secretion, one of the most reliable markers of circadian phase. Moreover, decreased sensitivity to morning light or a lengthening of the intrinsic period of the circadian clock was also associated with a delayed timing of sleep onset, although data supporting this hypothesis are

scarce. A forced desynchrony protocol by Carskadon and Acebo confirmed that sexual maturation was an important determinant in the timing of the circadian process C and that sexually mature adolescents were phase delayed, relative to prepubertal adolescents (Carskadon and Acebo, 2002). Taken together, there is convincing evidence that the phase delay during adolescence derives from a biological origin rather than from a loss of the ability to sleep due to school demands.

However, the reduction of TST on school days of 14 min per year across adolescence may not exclusively derive from changes in the circadian system but may also arise from changes in the homeostatic accumulation of sleep pressure. As previously introduced, SWA, a reliable marker of sleep pressure, remarkably declines between 11 and 17 years. This reduction of sleep pressure across adolescence may be the result of a reduced build-up of homeostatic sleep pressure during wakefulness with increasing age. This assumption received support by Jenni and colleagues who found a slower build up of sleep pressure in more mature adolescents (~14 years) compared to pre- or early pubertal adolescents (~ 12 years). In contrast, the dissipation rate was developmentally stable (Jenni and Carskadon, 2004). Thus, the slower accumulation of sleep pressure in more mature adolescents leads to lower sleep pressures in the evening allowing them to stay awake longer. Moreover, sleep onset latency, another marker of increased sleep pressure, was found to be shorter in younger adolescents compared to adolescents at older age (Taylor et al., 2005).

In summery, adolescent sleep is characterized by a reduction in TST mostly due to a delayed sleep onset time. This delayed sleep time might be a result of developmental changes in the sleep regulatory processes: The circadian clock delays and the build up of homeostatic sleep pressure is slowed down.

SLEEP DURING ADULTHOOD (26 TO 80 YEARS)

Changes in sleep duration and sleep architecture

Aging is a process of physical, psychological, and social change. Along with these aging effects, changes of sleep are a part of the normal aging process. Therefore, sleep at 25 years markedly differs from sleep in an 80 year old. Increasing age is often associated with a decrease in TST, an increase in sleep onset and, most significantly, a decrease in sleep efficiency. While adolescents exhibit a sleep efficiency of 90-95%, a 70-year-old subject only spends 80% of time in bed asleep (Ohayon et al., 2004). Across the same age span, sleep architecture changes towards a relative increase in superficial sleep (stage N1 and N2) at the cost of a decrease in deep sleep (stage N3). REM sleep slightly shifts to earlier nighttime sleep but its percentage remains relatively stable. Although stage N2 sleep becomes more prominent, its characteristics, spindles and K-complexes, become less numerous and the spindle frequency is slowed down (Wauquier, 1993). Sleep spindles, originating of an interplay between the thalamus and the cortex, are thought to be mechanistically involved in fostering long-term synaptic changes in the cortex. Indeed, several studies found a close relationship between parameters describing sleep spindles and the reinforcement of declarative memories (Born, 2010). The age-related change in sleep spindle parameters might therefore be related to the progressive decline in declarative memory in elderly. Moreover, a recent study investigating declarative memory performances in healthy elderly women found more spindles and higher spindle density in high compared to low performing older women (Seeck-Hirschner et al., 2012).

Once asleep, the hypnogram shows with increasing age more fragmented sleep or less consolidated sleep, characterized by frequent brief awakenings during night time sleep (Dijk et al., 2000). The progressive loss of sleep quality and quantity across age was associated with a higher prevalence of sleep disorders such as sleep apnoea, periodic leg movements during sleep or psychological disorders, like depression. However, despite all of these age-related variables, studies in healthy older adults without sleep disorders and sleep complaints reported similar declines in sleep quality and quantity (Ohayon et al., 2004). Thus, the reduction in sleep quality

and quantity is at least closely associated with an aging process per se rather than with ailments of aging.

Changes in EEG-spectral power

One of the most prominent sleep changes associated with aging between 26 and 80 is the progressive reduction of SWA after the age of 30. Thus, if SWA, as previously mentioned reflects synaptic density or strength of cortical synapses, one should expect that this substantial reduction in SWA across age is reflected in structural changes, i.e. a loss of synaptic density or strength. Indeed, such a decline of synaptic density is observable and was associated with an age-related decline in memory performance. Also in this association the declarative memory is particularly affected. Moreover, a recent MRI study supports a close relationship between age-related changes in spectral power and structural cortical alterations in elderly. The study found that age-related medial prefrontal cortex gray-matter atrophy predicted the extent of disrupted SWA in older adults. Moreover, the extent of the impairment of SWA was in turn associated with cognitive deficits (Mander et al., 2013).

Circadian and homeostatic changes of sleep

Not only does the quality and intensity of sleep change with increasing age, but the timing of that sleep also changes in the aging process suggesting that age-related changes in sleep may also be related to weaker circadian regulation. As mentioned above elderly sleep less and spend less time in bed mostly due to earlier wake up times compared to younger adults. However, not only do they wake up earlier but they also go to bed earlier. As a result of this shift in bed times they become more morning types (Fig. 4.1.4). This shift towards morning types goes along with changes in the circadian rhythms with increasing age. Healthy older adults compared to adults at younger age show a phase advance in their circadian rhythm of about 1 hour (Dijk et al., 1999). Moreover, across 24 hours elderly exhibit a reduced amplitude in the changes of core body temperature and melatonin secretion (Dijk et al., 1999, Munch et al., 2005). Therefore, aging was associated with a weakening of circadian regulation, which was also confirmed by studies showing

diminished melatonin secretion and a degeneration of the suprachiasmatic nucleus (SCN) in human post-mortem studies (Hofman and Swaab, 2006). However, determining the cause of the observed changes in the circadian system is difficult because aging is accompanied by many additional factors. For example, elderly are retired and thus less forced to maintain a daily routine which might be related to the weaker circadian regulation. On the other hand the lack of a daily routine is often accompanied by increased day time naps and/or reduced physical and mental activities, which leads to less sleep need at night and might be associated with difficulties falling asleep. Another important factor is the reduced exposure to external Zeitgebers, like exposure to the natural light-dark cycle, also related to poorer eyesight. More specifically, aging is often accompanied by reduced light transmission due to more opaque lenses and corneas (cataract). In particular short wave length light transmission known to play an important role in the entrainment of circadian rhythms is reduced in elderly (Brainard et al., 1997). Accordingly, cataract surgery has been shown to not only improve sight but in addition sleep quality (Tanaka et al., 2010).

The above mentioned longer sleep latency observed in older people combined with less SWA at sleep onset despite the increased wake duration at older age implies an altered homeostatic regulation of sleep. To investigate the homeostatic response to changes in sleep pressure, a study looked at the effects of reducing sleep need by means of daytime naps on post-nap SWA responses. Interestingly, both young (mean age, 22.4 years) and elderly (mean age, 71.4) subjects showed a similar homeostatic response of SWA suggesting that sleep homeostasis is not affected by age (Campbell and Feinberg, 2005). Other protocols manipulating sleep pressure found unchanged sleep homeostasis in elderly, despite the fact that these older volunteers had impaired sleep consolidation and reduced levels of SWA (Cajochen et al., 2006). Although the homeostatic response to changes in sleep pressure seems to be stable across adulthood, previous studies showed that the decline in SWS and SWA across the night is reduced in older subjects (Dijk et al., 1989, Landolt et al., 1996). As discussed previously is the separation of circadian and homeostatic processes difficult. A forced desynchrony study performed by Dijk et al. (Dijk et al., 1999) reported in healthy young (mean age, 26.7 years) compared to old subjects (mean age, 67.4 years) reduced sleep efficiency and SWS in older

people at all circadian phases of sleep. In addition, the same study reported an age-related reduction in the strength of the circadian drive for sleep, which is in line with more recent findings (Munch et al., 2005, Hofman and Swaab, 2006, Cajochen et al., 2006). Forced desynchrony studies also confirmed a shallower decline in SWS and SWA across the night (Cajochen et al., 2006, Dijk et al., 1999). However, whether the age-dependent changes in sleep structure and sleep consolidation depend on alterations of the circadian or the homeostatic system or its interaction is to date unclear. Thus, it is unknown if the observed changes in circadian parameters are due to SCN degeneration, different responses more “down stream” from the SCN or perhaps to aspects like reduced short-wave length light because of different lens properties. Similarly, it remains unknown whether the age difference in the decline of SWA across the night is related to changes in cortical functioning, which seem not to be related to the homeostatic regulation of sleep since this regulation remains stable during aging.

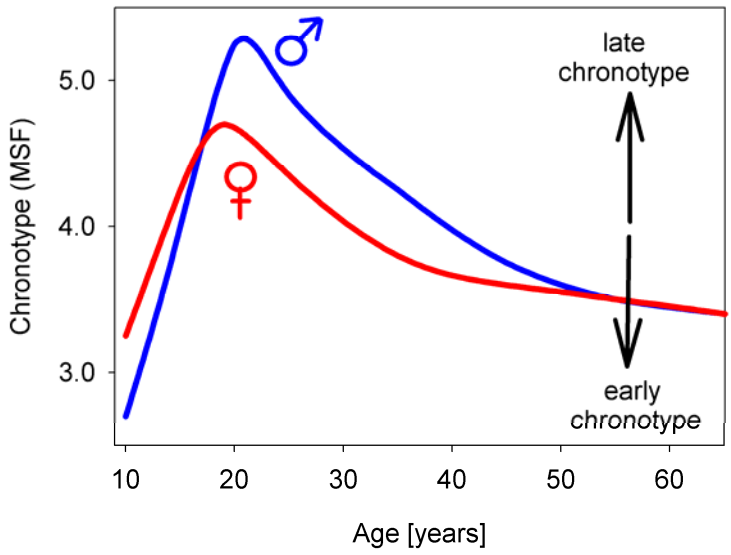

Figure 4.1.4: Chronotype fluctuations across life. Average chronotypes assessed by mid-sleep-point on free days (MSF) undergo maturational changes during puberty and adolescence. During puberty the average chronotype shifts from morning types to evening types, whereas during late adolescence and adulthood the preference shifts back to morning types. Note that girls with an earlier pubertal onset than boys

also shift earlier towards evening types and reach the maximal phase delay earlier than boys (redrawn from Roenneberg et al., 2004).

4.2 From sleep in humans to sleep in mice and rats

Sleep characteristics

Not only humans sleep. Sleep is known to be a universal state and assumed to have the same underlying vital functions in all species (Campbell and Tobler, 1984, Cirelli and Tononi, 2008). By means of electrographical recordings, the same 3 vigilance states can be discriminated in both humans and mammals: wake, REM sleep and NREM sleep (Zeplin, 2005). Exclusively in humans NREM sleep is further subdivided into stages 1 to 3. In contrast to humans, in rodents, the EEG or EMG electrodes are not attached to the scalp or the chin muscles, respectively, but chronically implanted on top of the cortex (and therefore termed electrocorticogram (ECoG)) or inserted in the neck muscles for EMG measurements. This ensures longitudinal electrophysiological measurements across 24 hours for several consecutive days or weeks. While in rodents the recording of ECoG and EMG is sufficient to discriminate between vigilance states, in humans, recording EOG is also necessary. Fig. 4.2.1 shows typical ECoG and EMG traces for the Sprague-Dawley rat. These traces exhibit characteristic voltage changes depending on the vigilance state similar to what is observed in humans (see Fig. 4.1.1). Briefly, wake is characterized by the presence of prominent muscle activity in the EMG, whereas the ECoG shows fast irregular activity of low amplitude. NREM sleep is characterized by low EMG while the corresponding ECoG exhibits big amplitude waves at low frequencies. REM sleep shows a similar low EMG as seen during NREM sleep, whereas the ECoG is dominated by regular theta activity.

Although the same three vigilance states can be distinguished in all mammals, the distribution of sleep within one day as well as total sleep time vary considerably between mammals and even between species (Zeplin, 2000). In contrast to humans who show a single main sleep episode of approximately 8 hours during the dark period, rats and mice show a polyphasic sleep pattern during which sleep predominates during the light period with a total sleep time in percentage of 24 hours between 50-65% (Franken et al., 1991, Borbely and Neuhaus, 1979). Despite the

variable sleep duration and its different distribution across 24 hours, sleep regulation in mammals is based on similar principles which are addressed in the next section.

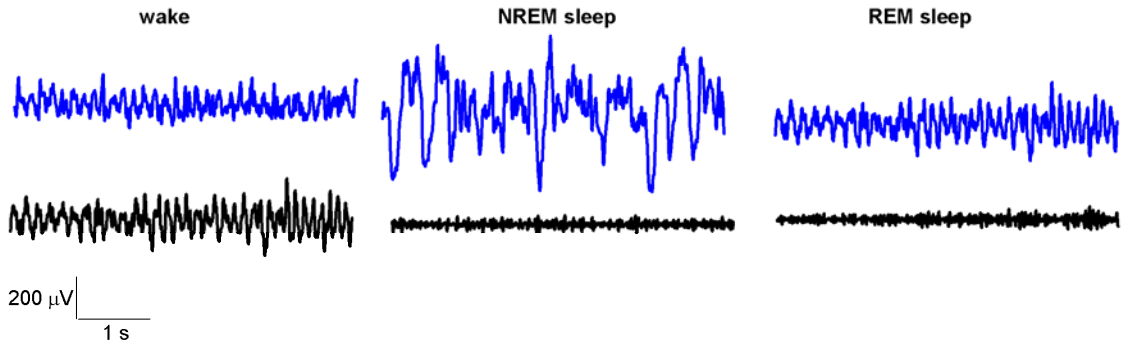

Figure 4.2.1: Electrocorticogram (ECoG) and electromyogram (EMG) traces of the Sprague-Dawley rat are illustrated for the 3 vigilance states waking, NREM sleep and REM sleep.

The regulation of sleep

As introduced in 4.1, sleep regulation in humans is best described by the two-process model, which relies on 3 main concepts. First, the circadian modulation of sleep is regulated by the SCN and ensures the correct timing of sleep within 24 hours. Second, prolonged wakefulness leads to intensified NREM sleep measured by an increase in SWA as a function of prior waking. Third, the two processes are thought to operate independently (Borbély, 1982). However, does this model also apply to mammals like rats and mice?

Since a direct retinal projection to the SCN, a pathway by which light information reaches the SCN, is common to all mammals (Moore, 1983), it was suggested that the SCN would regulate circadian rhythmicity in all mammalian species. This was confirmed by SCN lesion studies: SCN damage in humans (Cohen and Albers, 1991) as well as SCN lesions in primates (Albers et al., 1981, Reppert et al., 1981), rats (Stephan and Zucker, 1972) and mice (Ibuka et al., 1980) resulted in all species in the elimination of circadian rhythmicity.

Beside the circadian system, homeostatic regulation of sleep has also been tested in humans and various other species. In particular, rodents were extensively

studied by manipulating the amount of wakefulness prior to sleep (Borbely and Achermann, 1999, Tobler, 1995). These studies confirmed that longer periods of wakefulness led in general to intensified sleep, reflected by an increase in SWA. Another hallmark of the human EEG during NREM sleep is spindle activity (EEG power between 12 and 14 Hz). Several studies in humans reported an inverse relationship between spindle-activity or spindle-density and SWA (Aeschbach et al., 1997, Dijk et al., 1993, Uchida et al., 1991, Werth et al., 1997). Since SWA is highest at maximal sleep pressure, spindle density or activity is highest at low sleep pressure. This suggests that spindles are a hallmark of light sleep. This inverse change was also apparent after sleep deprivation (Borbely et al., 1981, Finelli et al., 2001). More recently, sleep spindles were quantified in mice (Vyazovskiy et al., 2004) and similar characteristics were found to what is known for humans: Spindles in mice are also a hallmark of NREM sleep, and in particular at NREM/REM transitions a period when sleep pressure is reduced. Moreover, spindle density increased in the course of the light period when SWA decreased, and decreased progressively after dark onset when SWA started increasing. Even more so, the performed sleep deprivation led to decreased spindle-density. Furthermore, there is evidence in both humans and mice that spindles are partly regulated by the circadian system unlike SWA (Vyazovskiy et al., 2004, Aeschbach et al., 1997, Dijk and Czeisler, 1995).

The third concept stipulates that the circadian and sleep-homeostatic processes operate independently. In humans, strong support for the independence of the two processes derives from forced desynchrony experiments, which allow separating the homeostatic and circadian facets of sleep (Dijk and Czeisler, 1994, Dijk and Czeisler, 1995). Similar results were found in rats where SCN lesion induced a disruption of the circadian sleep/wake distribution. However, longer periods of waking still led to an increase in SWA (Tobler et al., 1983, Trachsel et al., 1992, Mistlberger et al., 1983). Similar results supporting the independence of the two processes were obtained in the chipmunk (Dijk and Daan, 1989) and the Djungarian hamster (Tobler and Deboer, 1994). Although initially sleep homeostasis and the circadian process were thought to operate independently, more recent data indicate that they might not operate completely independent from each other. For example, clock genes thought to be mainly responsible for the correct timing of sleep within 24 hours were found to influence sleep homeostasis as well. For example, targeted disruption of core

circadian clock genes in mice affected sleep duration, sleep structure as well as SWA. The influence of clock genes is not restricted to mice but is also present in humans. Human polymorphisms in the PER3 gene, a well-known core clock gene altered electrophysiological markers of sleep homeostasis (for review, see Franken and Dijk, 2009).

To sum up, the two-process model regulating sleep is not only valid in humans but can be applied to rodents as well as to other species. Since it was proposed, this model has become an important conceptual framework frequently used in the interpretation of sleep studies in a wide variety of mammals. However, as introduced in 4.1, sleep regulation in humans is not fully developed at birth and undergoes further changes. Thus, the next section introduces how sleep regulation evolves during postnatal development in the rodent in comparison to what is known from human development.

The development of sleep regulation

Infants hardly show any evidence for a circadian rhythm at birth. Circadian rhythmicity develops within the first few months of life and is fully developed latest at 6 months of life (Mirmiran et al., 2003). The observation that infants are unable to maintain consolidated bouts of waking comparable to adult levels suggests a reduced sleep pressure tolerance in infants. At this age, selective (Anders and Roffwarg, 1973) or total sleep deprivation (Canet et al., 1989, Thomas et al., 1996) does not lead to intensified sleep (more SWA at sleep onset) but increases sleep duration. When exactly sleep deprivation leads to an adult-like compensatory response in terms of increasing SWA is still unknown.

Similar to infants, neonatal rats show comparable changes in sleep regulation during early life. At birth, no circadian organization of sleep and wakefulness can be detected although endogenous SCN rhythms can already be identified in the fetal period (Reppert et al., 1988a). Then during weaning, sleep in rats younger than P20 shows an increase in SWA across the light period. Only after P24, the SWA pattern follows an adult-like time course characterized by maximal SWA at light onset followed by a gradual decrease during the light period (Frank and Heller, 1997). Similar to the immature SWA pattern observed between P12 and P20, sleep

deprivation in that age span does not result in an adult-like response. Similar to what is observed in infants, sleep deprivation at this age has been shown to lead to a compensatory increase in sleep time (Frank et al., 1998). At P24, the same age when an adult-like time course of SWA can be observed, sleep deprivation first led to an increase in SWA (Frank et al., 1998). Thus, by the age of 24 days, rats show an adult-like organization in the distribution of sleep and wake, including a similar daily time course of SWA as well as a comparable response to sleep deprivation. The investigation of sleep pressure in slightly older animals, though mice, showed a similar mature homeostatic regulation of sleep during early adolescence (P19) and throughout adulthood. Nevertheless, sleep deprivation across age led to variable SWA rebounds in particular in younger mice. The strongest predictor for this variability in SWA rebound after sleep deprivation was the SWA decline during the light phase in baseline. Mice with high peak SWA at light onset, resulting in a large SWA decline were more likely to show no SWA rebound after sleep deprivation. Since under baseline SWA showed the same homeostatic changes at all ages, the author's concluded that sleep homeostasis has already matured at P19. The variable increase of SWA after sleep deprivation in younger mice was hypothesized to originate from a ceiling effect when wake is extended beyond its physiological duration (Nelson et al., 2013).

In summary, the regulation of sleep has been shown to be fully developed in humans at 6 months of age and in rats at 24 days of age. Similarly, in mice, compared to rats sleep regulation was found to be completed at a comparable age. Since the present thesis investigated sleep in rats older than 24 days, the research was performed during an age period at which sleep regulation is fully developed.

Electrocortical recordings (ECoG): What do we measure?

In the present thesis, a main focus was the analysis of ECoG recordings. This technique measures neuronal activity via electrodes placed directly on the exposed surface of the cerebral cortex. The ECoG measures extracellular field potentials as they changes in response to the transmembrane currents arising from neuronal activity with respect to a reference potential located above the cerebellum. All currents in the brain lead to intracellular as well as extracellular voltage deflections influencing the extracellular field measured with ECoG. However, their relative contributions differ and depend on the magnitude of the induced dipole that partly depends on the cytoarchitecture of a cell. Pyramidal cells feature a cell shape which considerably contributes to the extracellular field by the generation of strong dipoles. Another factor that substantially contributes to the magnitude of extracellular currents relies on temporal synchronous fluctuations of the membrane potential resulting in dramatically different magnitudes of measured field potentials (Buzsaki et al., 2012).

While wake and REM sleep exhibit similar ECoG traces, they strongly differ during NREM sleep (Fig. 4.2.2A). ECoG traces are based on neuronal activity and therefore the neuronal firing pattern is expected to be different in wake and REM sleep compared to NREM sleep. Accordingly, the neuronal firing pattern is similar between wake and REM sleep (Fig. 4.2.2B), in contrast to the neuronal firing pattern observed during NREM sleep. The latter is characterized by neurons alternating between prolonged hyperpolarized down states or OFF states lasting a few hundreds of milliseconds, during which neurons do not fire and depolarized up states or ON states, during which neurons exhibit sustained neuronal firing (Steriade et al., 2001, Timofeev et al., 2001, Vyazovskiy et al., 2009). This alternating pattern leads to high-amplitude voltage changes measured on the cortical surface (Fig. 4.2.2A).

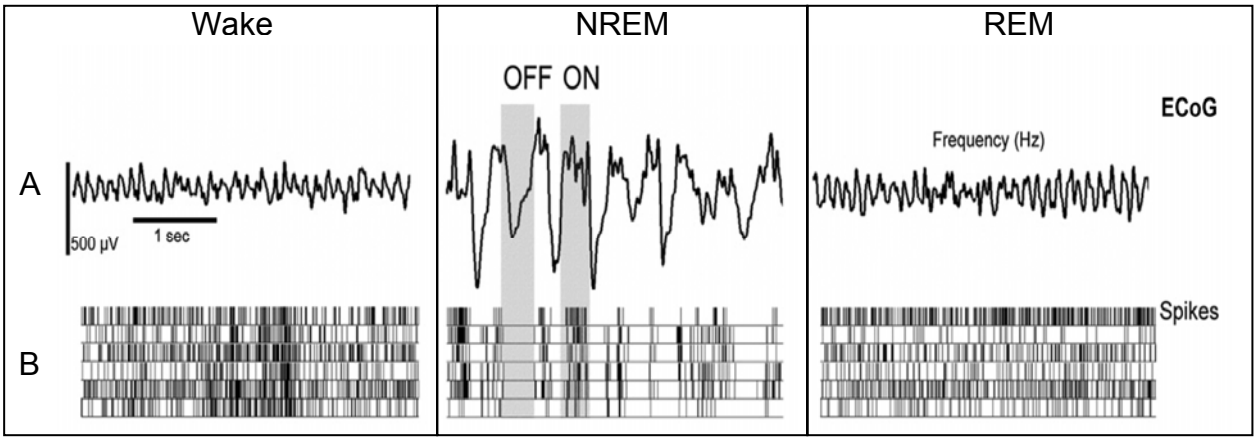

Figure 4.2.2: Neuronal activity during wake, NREM and REM sleep in the barrel cortex: **A)** Extracellular measured neuronal activity (ECoG) **B)** Raster plots of spike activity (each vertical line represents a spike whereas the horizontal lines represent different neurons) (adapted from Vyazovskiy et al., 2009).

ECoG analysis

The visual inspection of every 4 s epoch of the ECoG together with the EMG served to assign one of the 3 vigilance states. For an objective analysis of the ECoG, spectral analysis, which is a useful technique to quantify bioelectrical changes in the ECoG activity, was used. To perform spectral analysis the Fast Fourier Transformation (FFT) was used to transform the time-based ECoG into the frequency domain (Cooley and Tukey, 1965). Therefore, the digitized ECoG signal, a time series of data points sampled at equal interval, was decomposed into series of sine and cosine functions of varying amplitude and frequency (Geering et al., 1993). In the present study, the sampling rate was set at 128 Hz and each 4 s epoch was subjected to spectral analysis. These preset parameters define the properties of the transformed frequency based signal. With an epoch length of 4 s, a frequency resolution of 0.25 Hz is reached. Then, the sampling rate of 128 Hz limits the highest frequency contained in the recorded signal since the sampling frequency must be greater than twice the highest frequency contained in the recorded signal. Since most of the cerebral signal observed in the ECoG falls in the range of 1-30 Hz, a sampling

rate of 128 Hz covers the physiological range of neuronal activity. Activity below or above this range are likely to be artifacts.

Neuronal plasticity and SWA

The observation that the amount of prior waking, a brain state related to the acquisition of new information and thus associated with neuronal plasticity influences subsequent sleep (Borbély, 1982) could indicate that synaptic activity during waking affects subsequent sleep.

Indeed, in recent years, this observation has received support in a number of studies in Drosophila, rodents and humans. All showed a close relationship between synaptic plasticity processes and sleep, in particular SWA during NREM sleep (Bushey et al., 2011, Huber et al., 2004, Vyazovskiy et al., 2008, Hanlon et al., 2009). Molecular markers of synaptic potentiation such as increased GluR1-containing AMPA receptor (AMPAR) levels or the calmodulin-dependent protein kinase (CaMKII) were found to be increased after waking, while markers of synaptic depression were increased after sleep (Vyazovskiy et al., 2008). In a further study, neuronal activity was found to be increased in waking and decreased after sustained sleep (Vyazovskiy et al., 2009), suggesting that synaptic strength is upregulated in waking and downregulated during sleep. More evidence for a close relationship between wake and synaptic potentiation as well as sleep and synaptic depression has been found in humans by using high-density EEG. Huber and colleagues found an increase in SWA after performing a visuomotor learning task. This increase in SWA was restricted to the corresponding cortical areas presumably involved while learning the task (Huber et al., 2004). In contrast, when a procedure leading to synaptic depression was applied by means of arm immobilization for 12 hours, SWA over the involved cortical area was markedly reduced (Huber et al., 2006). Similar results were found in a study in which rats were trained in a reaching task which led to a post-training increase in SWA in the trained cortex. The SWA increase also correlated with performance changes in the reaching task (Hanlon et al., 2009). Taken together, these studies provide evidence for a close relationship between wake-related synaptic potentiation and an increase in SWA during subsequent sleep. Furthermore, sleep is related to the reduction of synaptic strength, a hypothesis

which was recently proposed to be a central function of sleep (Tononi and Cirelli, 2006). But how is an increase in synaptic strength associated with elevated levels of SWA during subsequent sleep?

Therefore, more recent studies explored changes of the neuronal firing pattern leading in the EEG or ECoG to either high or low levels of SWA. As previously introduced, during NREM sleep, virtually every cortical neuron engages in slow oscillations, consisting of up (sustained neuronal firing) and down states (neuronal quiescence) (Amzica and Steriade, 1998, Destexhe et al., 1999, Steriade et al., 1993b, Steriade et al., 2001). The observation that SWA is related to up and down states suggests that this alternating neuronal firing pattern changes in relation to prior waking. A more recent study investigated by means of cortical unit activity in freely behaving rats the cortical firing pattern after sustained wakefulness and sustained sleep (Vyazovskiy et al., 2009). Interestingly, the neuronal firing pattern changed as predicted. Sustained wakefulness was related to long down states or OFF states and short up- or ON states of synchronous firing. After sustained sleep synchrony decreased while the duration of ON periods increased.

In summary, SWA increases as a function of prior wakefulness and is associated with an increase in synaptic strength. This leads, at sleep onset, to a higher synchronous firing pattern reflected by high SWA levels. The synchronicity of neuronal firing across longer sleep periods decreases associated with a decrease in synaptic strength.

So far, I discussed the overall relationship between wakefulness and SWA and its relation to synaptic strength and neuronal firing patterns. However, the underlying cellular level processes responsible for changes in synaptic strength have not been addressed. This will be the topic of the next section.

Synaptic plasticity processes

During wakefulness, new information is acquired or learned and processed by electrical and chemical signal transmission via relays in the thalamus to the corresponding cortical area. A close relationship between learning, indirectly reflected by neuronal stimulation, and long-lasting changes in the corresponding network was already postulated in 1949 by Donald Hebb (Hebb, 1949). Donald Hebb

proposed that repetitive activation of pre- and postsynaptic neurons changes their synaptic properties. A huge body of evidence has confirmed long-lasting modifications due to neuronal activity at possibly every excitatory synapse summarised by the term synaptic plasticity (Malenka and Bear, 2004). The underlying mechanism is either long-term potentiation (LTP), leading to an increased postsynaptic response and therefore increased synaptic strength or long-term depression (LTD), characterized by a reduction in synaptic strength (Malenka and Nicoll, 1999).

At the cellular level the underlying mechanism for LTD/LTP induction requires the activation of postsynaptic N-methyl-D-aspartate (NMDA) receptors, a subtype of glutamate receptor. To do so, the postsynaptic cell needs to be depolarized. This is achieved by means of the activation of α-amino-3-hydroxy-5-methyl-4-isoaxalzolepropionic (AMPA) receptor, which channel is permeable to cations (Na^+ and K^+). Postsynaptic depolarization can lead to the activation of NMDA receptors, which exhibit a voltage-dependence because of the blocking of the channel pore by extracellular Mg^{2+}. During repetitive postsynaptic depolarization Mg^{2+} dissociates from its binding site within the NMDA receptor channel allowing Ca^{2+} entry into the cell. This is the trigger for LTD/LTP induction. Since postsynaptic rises in Ca^{2+} are needed for the induction of both LTD and LTP, it is assumed that particular properties of the Ca^{2+} signal may determine whether LTP or LTD is induced. Large increases in intracellular Ca^{2+} have been shown to induce LTP, while modest intracellular Ca^{2+} increase led to LTD and low calcium induced no plasticity (Lisman, 1989, Yang et al., 1999). This was confirmed by monitoring intracellular Ca^{2+} levels of stimuli normally inducing either LTP or LTD. Stimuli trains inducing LTP like high frequency or tetanus stimulation, led to higher intracellular Ca^{2+} levels with a slower rate of decay compared with stimuli inducing LTD (Hansel et al., 1997) evoked by low frequency stimulation. The underlying mechanism leading to variable postsynaptic Ca^{2+} influx has been proposed to depend on the order and timing of pre- and postsynaptic spikes (Markram et al., 1997). This dependence was first demonstrated by Bi and Poo (Bi and Poo, 1998) and is called spike-timing-dependent plasticity (STDP) (Song, 2000 and see for review Feldman, 2012). In short LTP occurs when presynaptic spikes lead postsynaptic spikes by up to ~20 ms, and LTD occurs when postsynaptic spikes lead presynaptic spikes by up to 20-100 ms, with a sharp (1-5

ms) transition between LTP and LTD. To induce plastic changes multiple pre-post spike pairs (60-100) are required (Bi and Poo, 1998, Markram et al., 1997, Debanne et al., 1998).

Intracellular Ca^{2+} levels then lead to long-lasting changes in synaptic transmission. High Ca^{2+} levels (induced via high frequency stimulation as tetanus stimulation) activate calmodulin-dependent protein kinase (CaMKII) that can subsequently phosphorylate many substrates including AMPA receptors (Barria et al., 1997) ultimately leading to a long-term increase in synaptic strength (Lledo et al., 1995). In contrast, a moderate increase in postsynaptic Ca^{2+} was shown to activate calcineurin, a key player in LTD induction by means of AMPA receptor dephosphorylation.

Synaptic plasticity is not only a key feature related to sleep and waking but also plays a pivotal role during cortical maturation. In the next section cortical maturation in humans, rodents and its relation to SWA is introduced.

SWA and cortical maturation

The human brain is not fully developed at birth and only corresponds to about 25% of its final adult mass (Robson and Wood, 2008). Thus, a relatively large proportion of brain size growth and reorganization takes place postnatally. Magnetic resonance imaging (MRI) studies in humans have shown that gray matter volume increases until the late childhood and then decreases during adolescence and young adulthood (Giedd, 2004, Giedd et al., 1999, Pfefferbaum et al., 1994, Shaw et al., 2006). These volumetric changes in gray matter are accompanied by synaptogenesis, in particular during early postnatal brain development. During this period, new synapses are formed with a developmental peak also in mid-childhood (Huttenlocher and Dabholkar, 1997, Liu et al., 2012, Huttenlocher, 1979). Subsequently, during puberty and adolescence synapses are getting pruned in part through activity-dependent processes (Hua and Smith, 2004). This extends into the third decade of life (Petanjek et al., 2011). During this process, weak or unused synapses are getting eliminated, in part to optimize cortical innervation but also to limit the brain's energy requirements as synapses are energetically very costly

(Harris et al., 2012). This prolonged neocortical development characterized by an inverted U-shaped trajectory of synapse density occurs throughout the cortex and is illustrated for the frontal cortex in humans in Fig. 4.2.3A (Huttenlocher and Dabholkar, 1997). This increase-decrease trajectory of synaptic density is not unique to humans but can also be observed in other primates (Bianchi et al., 2013, Liu et al., 2012, Rakic et al., 1986). During human development, changes in synaptic density were found to be paralleled by similar changes in SWA or in the amplitude of slow waves (Fig. 4.2.3B) (Feinberg, 1982, Huttenlocher, 1979, Huttenlocher and Dabholkar, 1997, Kurth and Huber, 2012). A possible explanation for the close relationship between SWA and synaptic density is provided by the observation that the amplitude of slow waves, the major contributing factor to spectral power in the slow wave frequency range, depends on the ability of a cortical network to synchronize its neuronal activity (Vyazovskiy et al., 2009). The speed of synchronized cortical activity depends on the strength and density of its connections (Esser et al., 2007, Vyazovskiy et al., 2009). Thus, the maximal synaptic density during mid-childhood results in maximal synchronization of cortical neuronal activity resulting in maximal amplitude slow waves in the surface EEG.

Like in humans, the rat brain is not fully developed at birth. For a long time, a marker of prenatal brain development was thought to be the amount of REM sleep in newborns relative to adult levels. As in humans, rats show high levels of REM sleep compared to adult levels indicating extensive postnatal brain maturation in both, humans and rats (Jouvet-Mounier et al., 1970). Moreover, in the neonate rat, individual laminae can only be differentiated after the first postnatal week (Eayrs and Goodhead, 1959). Both the rat and the mice cortices show dramatic changes in synaptic density during early development. They show a similar inverted U-shaped trajectory of synaptic density as found in humans. Initially, synaptic density progressively increases and peaks towards the end of the first postnatal month. It is then followed by a gradual reduction of mostly excitatory synapses (Nakamura et al., 1999, DeFelipe, 1997). This is illustrated for mice in Fig. 4.2.3C. The selective elimination of mainly excitatory synapses during puberty was also found in the primate cortex (Bourgeois et al., 1994). Moreover, the observed decrease in synaptic density in the rat was not only associated with a pruning process but also with a neuronal loss (Markham et al., 2007).

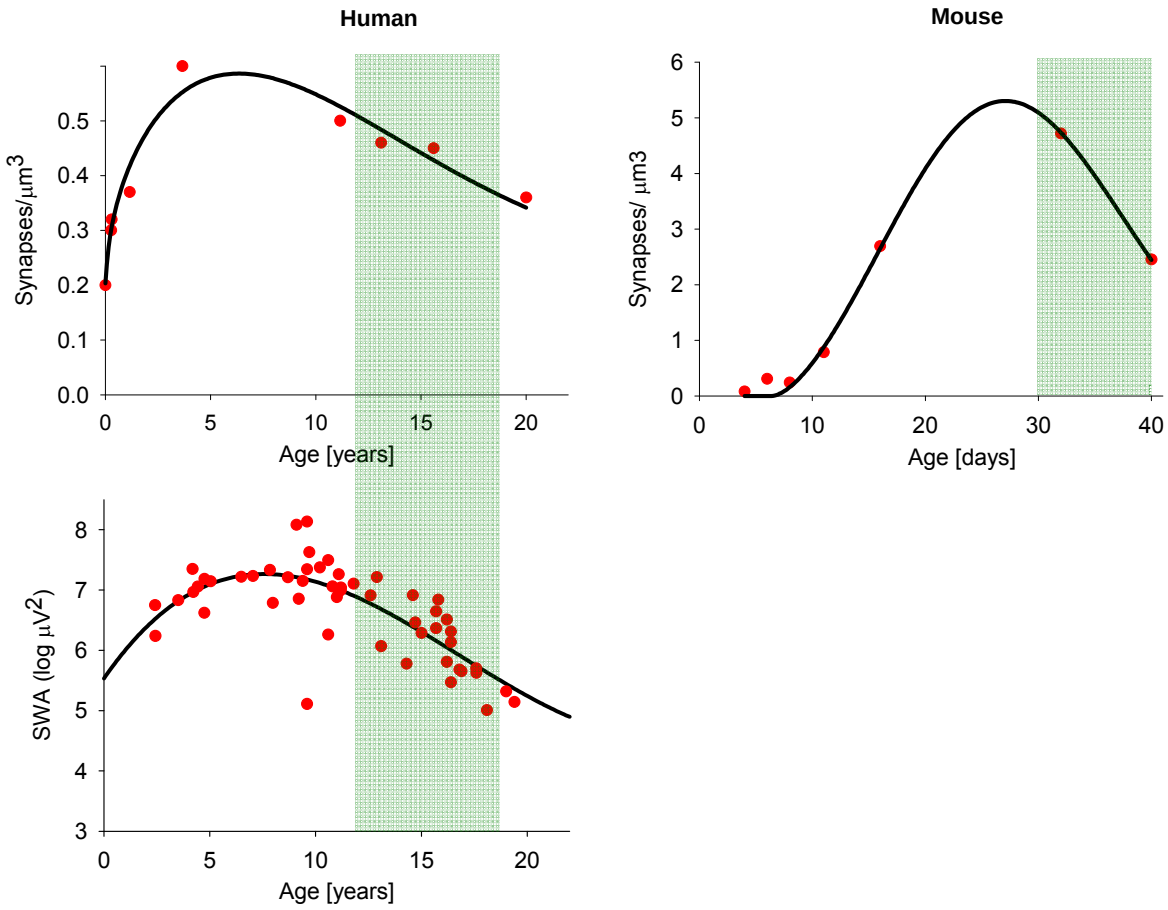

Figure 4.2.3: The developmental trajectories of synaptic density in humans and mice and of SWA (only in humans) **A)** Synapse density of the human frontal cortex is shown across age (redrawn from Huttenlocher and Dabholkar, 1997). **B)** Trajectory of SWA across age (redrawn from Kurth and Huber, 2012). **C)** Synapse density of excitatory synapses is shown for the somatosensory cortex across age as an example in mice (redrawn from DeFelipe, 1997). For illustrative purposes, the green bar illustrates the rough puberty time span in humans and mice.

Manipulating SWA

While numerous studies show a close relationship between waking activity and subsequent EEG activity in relation to plasticity processes (Diekelmann and Born, 2010) fewer studies investigated the causality between these factors. A direct relationship between sleep and wakefulness can be addressed in protocols manipulating sleep or wakefulness, respectively. For example the manipulation of synaptic processes during wakefulness by means of a visuomotor learning task led to a local increase of SWA which was positively correlated with the performance improvement the next morning (Huber et al., 2004). On the other hand, manipulating sleep by slow wave deprivation via acoustic stimuli hindered sleep-dependent performance gains of visuomotor learning (Landsness et al., 2009). Slow waves can also artificially be boosted with oscillating transcranial direct current stimulation (tDCS). Applying this technique allows stimulating the brain with a desired frequency and entraining neuronal firing to a specific firing frequency (Frohlich and McCormick, 2010, Ozen et al., 2010). By using tDCS two studies showed that slow oscillations during NREM sleep are causally involved in the enhancing effect of sleep on memory consolidation (Marshall et al., 2006, Marshall et al., 2004). These studies showed that tDCS increased SWA in contrast to sham stimulation and led to an improved memory recall in a previously learned task. In summary, there is good evidence for a causal involvement of sleep, in particular sleep SWA, on memory performance which in turn is associated with synaptic plasticity.

A further way to manipulate sleep in one way or the other is via a pharmacological approach (Pagel, 2001). The administration of specific drugs to humans or animal models can induce reliable changes in sleep architecture and/or spectral power and provide an interesting tool to manipulate sleep and investigate its effects. If the molecular and cellular targets of the drug are carefully studied, this approach can provide insights into the underlying neurochemical mechanisms. One such drug that has been extensively studied in the past and was shown to induce effects on sleep similar in humans and rats is caffeine (Landolt et al., 1995a, Landolt et al., 2004, Landolt et al., 1995b, Schwierin et al., 1996). Several studies have shown that caffeine reduces the build up of SWA and caffeine application can thus be used as a further method to manipulate SWA to investigate a causal relationship between SWA and synaptic plasticity.

The close relationship between synaptic plasticity and SWA can also be investigated under pathophysiological conditions. For example, conditions like mood disorders are characterized by altered synaptic plasticity that may affect SWA and thus SWA may be used as a tool to investigate plasticity not only during development or adulthood but also in disease.

Depression/stress, plasticity and SWA

Neuronal plasticity is the ability to acquire information at different levels such as sensory, cognitive, emotional, and social or a combination of them to make appropriate responses to the same or related future stimuli. Therefore, it is likely that plasticity or remodelling also plays a significant role in the pathophysiology and treatment of major psychiatric illnesses, such as mood disorders. To date, preclinical and clinical studies support this hypothesis, demonstrating structural alterations in depressed patients. For example brain imaging studies in humans suffering from major depression report a reduction in hippocampal volume (Bremner et al., 2000, Sheline et al., 1999). Studies also report alterations in the cerebral cortex of patients with depression or bipolar disorder. These include a decrease in volume of the prefrontal cortex and a decrease in the number of neurons and glia (Rajkowska et al., 1999, Drevets et al., 1997). In humans, a major aetiological factor for the development of major depression is the exposure to chronic psychosocial stress (Kendler et al., 1999). Animal models of depression have made use of the proposed causal relationship between chronic stress and depressive-relevant behavior (Kudryavtseva et al., 1991, Golden et al., 2011, Azzinnari et al., submitted). Like in humans, studies in rodents report structural alterations in response to stress. For example, it has been shown in rats that psychosocial stress resulted in atrophy (Magarinos et al., 1996, Watanabe et al., 1992) and death of CA3 pyramidal neurons (Mizoguchi et al., 1992) as well as reduced neurogenesis of dentate gyrus granule neurons in the hippocampus (Tanapat et al., 2001). More recently, structural alterations were also found in the prefrontal cortex (Cook and Wellman, 2004). In line with the observation of reduced neuronal and/or synaptic plasticity related to depression and/or stress, chronic antidepressant treatments were shown to induce opposing effects. For example, a variety of chronic antidepressant treatments

belonging to the class of monoamine oxidase inhibitor (MOAI), norephinephrine reuptake inhibitor or selective serotonin reuptake inhibitor (SSRI) were shown to increase neurogenesis in rats (Malberg et al., 2000). Moreover, a very recently performed study investigated synaptic plasticity by means of postsynaptic density protein-95 (PSD-95), brain-derived neurotrophic factor (BDNF), synaptophysin (SYP) and dendritic outgrowth in relation to different classes of antidepressant drugs in the rat hippocampus. The study revealed that several SSRI's enhanced synaptic protein levels and encouraged dendritic outgrowth (Seo et al., 2013). Thus, there is evidence that depression or stress is related to impaired plasticity whereas a reduction of depressive symptoms can be achieved by the induction of synaptic plasticity through medical treatment.

Another antidepressant treatment, however only short-lasting but with a rapid improvement of depressive symptoms, was shown to be sleep deprivation (SD) (Wu and Bunney, 1990). Cross-sectional data showed substantial positive responses after an entire night of SD in close to 60% of totally 1'700 depressed patients of all diagnostic subcategories. The underlying mechanism of the beneficial effect of SD is however still unknown. Similar to antidepressant medical treatment, SD may be beneficial based on similar principles. Wakefulness is also associated with an increase of molecular markers of LTP (Vyazovskiy et al., 2008). Prolonged wakefulness leads to an overall increase in LTP and therefore would increase plasticity in depressive patients. In line with reduced plasticity in depression, it has been proposed that wakefulness in depression leads to a reduced build-up of SWA (Borbely, 1987). This is known as the S-deficiency hypothesis. Related to the close relationship between SWA and synaptic plasticity processes, this suggests that plasticity is impaired in subjects suffering from depression. Accordingly, SD is assumed to be therapeutic because it restores SWA back to normal levels.

Thesis aims

The overall aim of the thesis was to examine the homeostatic regulation of sleep during development, adulthood as well as in disease by means of ECoG. To do so, sleep homeostasis was investigated by means of SWA measurements. Based on increasing evidence showing a close relationship between synaptic plasticity and SWA, SWA was used as a marker for synaptic plasticity. By manipulating SWA, a potential role of SWA in development was investigated. Specific aims are formulated below. For each age period, one intervention known to alter SWA was investigated.

(1) Maturational processes and their manipulation (Sprague-Dawley rat)

Hypothesis: Analogous to age-related changes of SWA in humans, Sprague-Dawley rats show age-related changes in SWA in a comparable developmental period. These age-related changes in SWA are accompanied by further structural and behavioral markers of maturation. Second, if SWA plays an active role during maturation, its manipulation should lead to altered maturational markers.

To assess maturation in the rat, longitudinal ECoG recordings combined with structural and behavioral readouts were assessed between postnatal day 25 (P25) and P45, a developmental period corresponding to pre-puberty and puberty. The relationship between synchronous firing and SWA suggests that the more synchronous neurons fire, the more SWA can be measured (Vyazovskiy et al., 2009). A higher synchronicity can also be reached by a better-connected neuronal network, which predicts a close match between the developmental trajectory of synaptic density and SWA. This parallel inverted U-shaped trajectory of SWA and synaptic density was repeatedly observed in humans (Feinberg, 1982, Huttenlocher, 1979, Huttenlocher and Dabholkar, 1997, Kurth and Huber, 2012). Since, like in humans, the rat cortex during pre-puberty and puberty undergoes an inverted U-shaped trajectory of synaptic density (Nakamura et al., 1999), SWA is expected to follow a similar trajectory. Furthermore, SWA was proposed to play an active role in synaptic renormalization processes (Tononi and Cirelli, 2006) and thus might also play a role during development. We manipulated SWA by means of caffeine administration, a

substance known to reduce SWA in humans as well as in rats (Landolt et al., 1995a, Schwierin et al., 1996). If SWA plays an active role during maturation, we expect its manipulation to lead to altered maturational markers during development.

(2) Diurnal SWA changes across 24 hours (Sprague-Dawley rat)

Hypothesis: SWA across 24 hours does not follow an adult-like time course but exhibits features of additional maturational processes during development.

Waking leads to an increase in SWA associated with an increase in synaptic strength whereas during sleep, SWA declines, a decrease that is related to a reduction of synaptic strength (Vyazovskiy et al., 2008). In adulthood, these increases and decreases of SWA across 24 hours are in balance across days (Borbély and Achermann, 2005) which suggests a balance of synaptic plasticity processes across time. However, during pre-puberty and puberty, synaptogenesis and pruning is not in balance (Nakamura et al., 1999). We thus expected SWA to not follow an adult-like time course across 24 hours.

To address this question, we examined changes in SWA across 24 hours in the same data set as in (1) for specific postnatal days that are predominantly associated with synaptogenesis or pruning, respectively. To specifically relate SWA changes across 24 hours to the developmental period, the same analysis was performed in older and thus more mature rats. At this age, we expected a more adult-like time course of SWA.

(3) SWA in a mouse model of depression (Black 6 mouse)

Hypothesis: The time course of SWA across 24 hours is altered in a mouse model of depression.

A new mouse model of depression has recently been developed in the laboratory of PD Christopher Pryce (University Clinic of Psychiatry in Zurich). To examine the effect of psychosocial stress on sleep immediately after the induction of

chronic stress, the ECoG was recorded during a 24 hours baseline followed by a 4 hours sleep deprivation and a 20 hours recovery period.

In humans, a close relationship between sleep disturbances and depression is well known (ICD-10, 1994 , DSM-5, 2013). The observation that sleep deprivation leads to a reduction of depressive symptoms (Wu and Bunney, 1990) suggests that a wake-dependent process is slowed down in depressive patients and only reaches normal levels after prolonged wakefulness. The S-deficiency hypothesis proposes that SWA accumulates at a slower rate in people suffering from depression. Accordingly, SWA only reaches normal levels after prolonged waking (Borbely, 1987). Based on the close relationship between SWA and synaptic plasticity, synaptic plasticity processes may also be impaired in patients suffering from depression.

Based on the proposed impaired homeostatic accumulation of SWA in depressive patients (Borbely, 1987) and on the evidence for impaired cortical plasticity in chronically stressed rodents (Cook and Wellman, 2004) and humans suffering from depression (Drevets et al., 1997, Rajkowska et al., 1999), we expected reduced SWA levels in mice after they underwent a chronic stress protocol when compared to controls.

5 Experimental research part

5.1 The effects of caffeine on sleep and maturational markers in the rat

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ABSTRACT

Adolescence is a critical period for brain maturation during which a massive reorganization of cortical connectivity takes place. In humans, slow wave activity (< 4.5 Hz) during NREM sleep was proposed to reflect cortical maturation which relies on use-dependent processes. A stimulant like caffeine, whose consumption has recently increased especially in adolescents, is known to affect sleep wake regulation.

The goal of this study was to establish a rat model allowing to assess the relationship between cortical maturation and sleep and to further investigate how these parameters are affected by caffeine consumption. To do so, we assessed sleep and markers of maturation by electrophysiological recordings, behavioral and structural readouts in the juvenile rat. Our results show that sleep slow wave activity follows a similar inverted U-shape trajectory as already known in humans. Caffeine treatment exerted short-term stimulating effects and altered the trajectory of slow wave activity. Moreover, caffeine affected behavioral and structural markers of maturation. Thus, caffeine consumption during a critical developmental period shows long-lasting effects on sleep and brain maturation.

INTRODUCTION

Caffeine is the most widely consumed stimulant. It is known that caffeine consumption, in particular among adolescents, has increased significantly in recent years (Temple, 2009). The major effect of moderate caffeine consumption in the central nervous system is to block adenosine A1 and A2A receptors (Fredholm et al., 1999), which are present in almost all brain areas (Goodman and Synder, 1982, Fastbom et al., 1987). As a stimulant, caffeine has clear wake-promoting effects (Dunwiddie and Masino, 2001). Several studies have shown that caffeine diminishes the build up of sleep pressure during wakefulness (Schwierin et al., 1996, Landolt et al., 1995a, Fredholm et al., 1999). Thus, after caffeine application, the major electrophysiological marker of sleep pressure, EEG slow wave activity (SWA, <4.5 Hz) during non-rapid eye movement (NREM) sleep was reduced (Borbely and Achermann, 1999). In contrast, adenosine agonists seem to promote sleep, in particular NREM sleep (Benington et al., 1995, Schwierin et al., 1996, Ticho and Radulovacki, 1991). Together these studies show that caffeine has significant impacts on sleep wake regulation.

Adolescence is a critical period for brain development which is characterized by extensive morphological and functional changes (Johnson, 2001). In humans it was shown that maximal cortical synapse density is reached shortly before puberty, followed by a reduction in synapse density during adolescence (Huttenlocher and Dabholkar, 1997). These massive changes in connectivity are based on an overproduction of synapses during early development (DeFelipe, 1997), which is followed by a net elimination of synapses (Zuo et al., 2005). Such processes of cortical reorganization have been linked to activity-dependent processes (Hua and Smith, 2004). Studies show that behavior-dependent neuronal activity is needed for cortical maturation (Chattopadhyaya et al., 2004, Feldman and Knudsen, 1998).

Interestingly, the inverted U-shaped trajectory of synapse density is paralleled by changes in SWA (Frank et al., 2012, Kurth et al., 2010, Feinberg, 1982). In humans, SWA increases in pre-pubertal children, reaches a maximum around puberty and decreases during adolescence. A convincing explanation for the relationship between SWA and synapse density comes from our knowledge about

how slow waves are generated. SWA during NREM sleep is characterized at the cellular level by a specific neuronal firing pattern consisting of an alternation between a depolarized up state, when neurons keep firing, and a hyperpolarized down state, when neurons are silent. Intracellular recordings have shown that during deep NREM sleep virtually every cortical neuron is included in such slow oscillations (Steriade et al., 1993a, Steriade and Timofeev, 2003). Furthermore, a study by Vyazovskiy et al. (Vyazovskiy et al., 2009) discovered by means of multi-unit recordings in the rat a close relationship between sleep pressure and the synchronization of cortical neuronal activity during sleep. More specifically, the wakefulness-related increase in the amplitude of slow waves, as reflected by more SWA on the surface EEG, was associated with a more synchronous firing pattern. In the course of subsequent sleep neuronal synchrony was reduced. Interestingly, measures of synaptic strength follow a similar time course, i.e. show a net increase during wakefulness and decrease during sleep (Vyazovskiy et al., 2008, Huber et al., 2013). As numerous studies have shown is increased synaptic strength associated with an increased level of neuronal activity synchronization (Whitlock et al., 2006). These observations may suggest that changes in synaptic connectivity affect the level of synchronization which relates to corresponding SWA changes. All together these studies support a close relationship between cortical plasticity and SWA. Since cortical maturation involves vast plasticity processes, which are use-dependent, the question arises whether the application of a stimulant during this critical period affects the relationship between sleep SWA and cortical plasticity. To address this question the aim of this study was 1) to establish a juvenile rat model allowing to investigate the relationship between brain maturation and sleep, 2) to assess the effects of caffeine on sleep in these juvenile rats, and 3) to test whether such a caffeine application affects markers of brain maturation.

We performed longitudinal electrocortical recordings (ECoG) in 28 male Sprague-Dawley rats. In addition, we assessed behavioral and anatomical development by means of behavioral testing and immunohistochemistry, respectively. Our results show a similar trajectory of SWA in the rat as already found in humans. Moreover, caffeine intake during the period when under sham condition the sleep SWA trajectory started to decrease resulted in a delay of all three assessed markers of brain maturation.

METHODS

Surgical procedures

Animal protocols followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and facilities and were approved by the Cantonal Veterinary Office of Zurich.

Animals were delivered after weaning with 22 days of age. To immediately acclimatize the animals to the recording box all animals were placed in their box the day they arrived, 3 days before the recording started. Then, surgery was performed in 23- to 25-day old male Sprague-Dawley rats according to a protocol published previously (Franken et al., 1991). Under isoflurane anesthesia all animals were implanted epidurally with gold-plated miniature screws (0.9 mm diameter) for electrocortical recording (ECoG) [right hemisphere: frontal, 1.5 mm anterior to bregma, 2 mm lateral to the midline and parietal, 2 mm anterior to lambda, 2 mm lateral to midline; reference: above cerebellum, 2 mm posterior to lambda, on the midline]. Two gold wires (0.2 mm diameter) were inserted bilaterally into the neck muscles for electromyogram (EMG) recording. The electrodes were connected to stainless-steel wires and fixed to the skull with dental acrylic cement. All animals received a single dose of postoperative analgesic Temgesic (0.1 mg/kg Buprenorphin, s.c.) during the last 30 min of the surgery. After the surgery no animal lost weight, instead all animals showed normal weight gain across the experiment. At the end of the experiment all brains were carefully inspected and no cranial damage was observed. Histological analyses at the location of the previously positioned screws did not show any abnormalities.

Electrocortical recordings

For longitudinal recording the rats were connected by a fine cable to a swivel and remained connected throughout the experiment. Data collection started immediately after surgery for 20 consecutive days. Animals were singly housed and kept under a 12-h light (9 AM to 9 PM) and 12-h dark period. Food and water were given ad libitum. The ECoG and EMG signals were amplified (amplification factor,

~2000), filtered (highpass filter: -3 dB at 0.016 Hz; low-pass filter: -3 dB at 40 Hz) sampled with 512 Hz, digitally filtered [ECoG: low-pass finite impulse response (FIR) filter, 25 Hz; EMG: bandpass FIR filter, 20-50 Hz], and stored with a resolution of 128 Hz. The ECoG power spectra were computed for 4 s epochs by a fast Fourier transform routine. Adjacent 0.25 Hz bins were averaged into 0.5 Hz (0.25-5 Hz) and 1.0 Hz (5.25-25 Hz) bins. Before each recording, the EEG and EMG channels were calibrated with a 10 Hz, 300 μV peak-to-peak sine wave.

Vigilance states (NREM, REM and wake) were visually determined, as previously documented (Franken et al., 1991), by off-line visual inspection of the ECoG and EMG signals. Epochs containing artifacts in one derivation were excluded from spectral analysis of both ECoG derivations. As a representative time window the first 3 hours after light onset were used to determine vigilance states for every day. In addition, vigilance states for the entire 24 hours were determined for selected days throughout the longitudinal recordings: postnatal day 29 (P29)-P31, P35 and P38. Vigilance states could always be determined. Sample EEG traces are presented to indicate age and condition-dependent changes. To do so, the 4-s epoch scored as NREM sleep that exhibited highest SWA at the beginning of the light period was chosen for a representative sham and caffeine treated animal. Data analyses and statistics were performed using the MATLAB software package (MathWorks. Contrasts were tested by post-hoc parametric or non-parametric statistical tests after significance by ANOVA.

Caffeine application

We performed a pilot experiment to assess caffeine consumption and its dose-dependent effect on locomotor activity. We were aiming for a caffeine dosage which would minimally affect the amount of wakefulness but rather affect SWA during a specific time interval. Thus, in the pilot experiment we administered 3 different caffeine dosages via the drinking water (0.00 g/l, 0.15 g/l and 0.3 g/l). As in the main experiment, in this pilot experiment caffeine was administered between P30 and P34 during 8 hours per day from 6 PM to 2 AM. This time window contained the last 3 hours of the light period and the first 5 hours of the dark period. We chose this time window since we aimed at having maximal caffeine concentration at the beginning of

the dark period when the animals are naturally awake and leave enough time in the second half of the dark period for a wash out. With this approach we were hoping 1) to induce as little additional wakefulness as possible and 2) with a half life time ~1h (Fredholm et al., 1999) to have as little caffeine in the circulation as possible once the main sleep period starts (at the beginning of the light period). We performed actimetry but no ECoG in these pilot animals. All water bottles were quickly exchanged at these two time points and water and caffeine consumption was calculated based on the weight reduction of the water bottles. The results of the pilot experiment showed that overall locomotor activity across 24 hours was increased on the first day of caffeine treatment between the low caffeine consumers (0.15 g/l) compared to sham treated animals. However, at this dosage, during the remaining 4 days of caffeine treatment no changes in general locomotor activity was found. Based on this result, the same protocol was applied in the main experiment using the lower caffeine dosage (0.15 g/l, n=11 animals). In addition, based on the consistent and stable daily liquid intake across 8 and 16 hours found during our pilot experiment we expected similar caffeine consumption in our main experiment. Indeed, caffeine consumption did not differ between our pilot and the main experiment. In our main experiment water or caffeine consumption did not differ across 8 hours or 16 hours or across the entire 24 hours. The overall daily liquid intake was 24.5 ± 1.6 ml / 100 g body weight in sham treated animals and 24.8 ± 1.6 ml / 100 g body weight in caffeine treated animals. This is in line with a previous study showing a daily water intake of ~26 ml/100g body weight at the age of the 5th postnatal week (McGivern et al., 1996).

Maximal daily caffeine intake was calculated based on the rat's weight (at 6 pm) and resulted in a mean caffeine intake of 16.0 ± 1.4 mg/kg per day. Because caffeine metabolism differs between rodents and humans, this dosage corresponds to ~5 mg/kg in humans (Fredholm et al., 1999), which is about 3 to 4 cups of coffee (each containing ~100 mg caffeine) (Fredholm et al., 1999). The same procedure of changing water bottles twice a day and daily weighting was applied in sham treated animals (n=17). Experiments of caffeine and sham treated animals were run in parallel.

Behavioral tests

A free exploration task was performed in a subset of 8 sham and 9 caffeine treated rats at the ages P28 and P42 between 4.30 pm and 5.30 pm. During the test phase, each rat remained in its home cage and was exposed to a new object for 1 hour and its behavior was video recorded (Roline, RIC-45 IP Camera). The same procedure was repeated at an older age (P42) with a new object. The objects were counter-balanced among individuals. Offline, the predominant occurred behavior (quiet waking, grooming, exploring, object exploring) was determined for each 10 s interval for 1 hour per rat. In the results the 4 different behaviors are expressed as a percentage of the sum of all intervals with a behavior.

Immunohistochemistry

Based on evidence in non-human primates that the entire cerebral cortex matures as an integrated network rather than as a system-by-system cascade (Lidow et al., 1991), we expected similar changes in the different neurotransmitter systems. Previous publications in the rat showed extensive maturational processes in the dopaminergic and in the cholinergic system during the preadolescent period (Andersen et al., 2000, Gould et al., 1989). Moreover, there is good evidence suggesting a direct involvement of the cholinergic system in cortical development (Bruel-Jungerman et al., 2011, Guizzetti et al., 2008, Lohmann et al., 2002, Zhu and Waite, 1998). For example, cholinergic innervation is needed for the correct maturation of cortical barrels, which relies on plasticity processes (Zhu and Waite, 1998). Thus, cholinergic terminals seem to be a representative marker for overall cortical development. However, the development of different neuromodulators may vary in time and space (Cohen and Neff, 1982, Pedata et al., 1982) and, as a consequence, may show a deviation from overall cortical development. With this limitation in mind we used the changes in the cholinergic system across age as a marker of cortical maturation. Therefore, we performed immunohistochemistry using DAB immunoperoxidase reaction to assess the vesicular acetylcholine transporter (VAChT) protein, mostly present in presynaptic terminals. To do so, a subset of different animals was perfused at two time points P30 and P42 either after sham or caffeine treatment (n=6 per group). The animals were deeply anesthetized with an

overdose of isoflurane and perfused through the ascending aorta with PBS (pH 7.4), followed by 4% paraformaldehyde in PBS. Then the brain was removed from the skull and postfixed overnight in the same fixative solution.

Afterwards the brains were rinsed in PBS, cryoprotected in 30% sucrose solution in PBS and frozen at -80 °C. Coronal sections were then cut from frozen blocks with a sliding microtome (40 µm) and stored at -20 °C in anti-freeze. Further processing was performed according to a protocol published previously (Fritschy et al., 1998). To label VAChT, presumably reflecting cholinergic presynaptic terminals, the sections were incubated overnight at 4°C in the primary antibody solution containing rabbit-anti vesicular acetylcholine transporter (VAChT, Nr. 139103, 1:1000, Synaptic Systems, Goettingen, Germany) in PBS containing 2% normal goat serum and 0.2% Triton X-100. The next day the sections were processed for immunoperoxidase labelling. To do so, all sections were incubated for 30 min at room temperature in biotinylated secondoary antibodies (diluted 1:300 in Tris-saline containing 2% normal goat serum) and processed for avidin-peroxidase staining (Vectastain Elite Kits, Vector Laboratories, Burlingame, CA). Up to 5 sections were kept in one well and stained for 5 to 10 minutes. After three washes in Tris-saline, sections were mounted on gelatinized glass slides and air-dried overnight.

Quantification of immunohistochemical staining

The immunoperoxidase-stained slides were scanned with a slide-scanning microscope (Mirax Midi Slide Scanner; Zeiss) in bright-field mode at the level of the corpus callosum (Bregma 1.70 to 1.60). Images were acquired with a digital camera (1288 x 1040 pixels, with a pixel size of 0.23 um; AxioCam monochrome charge-coupled display; Zeiss) with a 20x objective (NA 0.8) using the software Pannoramic Viewer (version 1.14.25.1; 3D Histech Ltd, Budapest, Hungary). For quantitative analysis the ImageJ software was applied (Version 1.45s; NIH, USA) to export 6 randomly selected high-magnification JPG images, 3 in each hemisphere of the primary somatosensory cortex in layer II and III. All analyses were performed blinded related to age, condition and image. Each of the selected images was then converted into an 8-bit image. As done routinely, to correct for staining intensity each image was treated individually and a threshold was determined manually (Krstic et al.,

2012). For further analyses the VAChT stained area of each image was expressed as percentage of total area.

RESULTS

Sleep slow wave activity in juvenile rats shows an inverted U-shape trajectory as found in humans

For the assessment of age-dependent changes in sleep, we longitudinally recorded electrocortical recordings (ECoG) in the Sprague-Dawley rat after weaning, starting on postnatal day 25 (P25) for 20 consecutive days. First, we examined age-dependent changes of the vigilance states for selected days. We found an increase in wakefulness with increasing age during the light and the dark period, which was paralleled by a decrease in NREM sleep (Fig. 5.1.1).

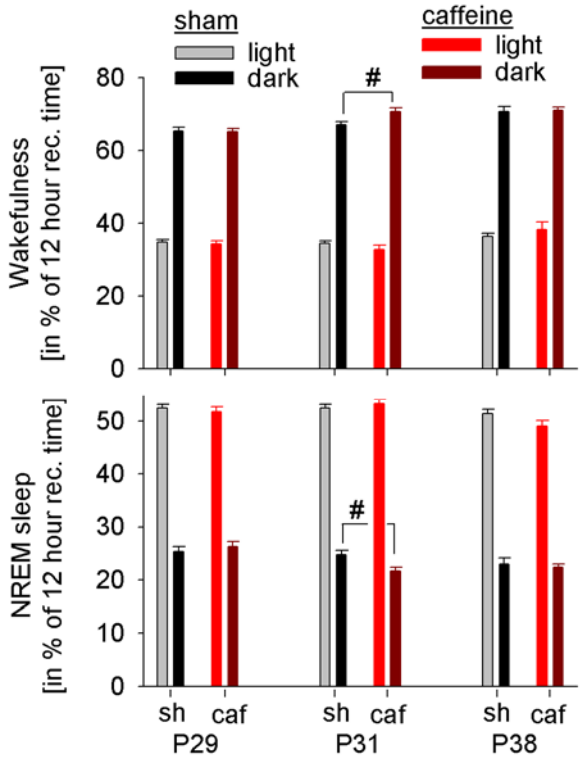

Figure 5.1.1: Vigilance states across age. Wakefulness and non rapid eye movement (NREM) sleep in sham (sh, n=15) and caffeine (caf, n=11) treated animals, expressed as a percentage of 12 hours recording time (rec. time) for the light and dark period before (P29), during (P31) and after (P38) caffeine treatment. A two-way repeated measures ANOVA with factor age (P29, P31 and P38) and condition (caffeine and sham) performed for NREM sleep and wakefulness during the light period was significant for age. The same analyses for NREM sleep and wakefulness during the dark period revealed an effect of age and an interaction between condition and age (all, $p<0.05$). The group comparison during caffeine application (P31) showed increased wakefulness and decreased NREM sleep during the dark period, respectively (# $p<0.05$, unpaired Student's t-test).

Next, we quantified age-related changes in the ECoG by performing spectral analysis. We focussed on sleep slow wave activity (SWA, ECoG power between 1 and 4 Hz during NREM sleep, averaged across the first 3 hours after light onset), as it was shown to exhibit prominent age-dependent changes in humans. SWA in the rat increased progressively during early development and reached a plateau during puberty (P30), before declining significantly during subsequent days (Fig. 5.1.2B). A comparison of the ECoG spectrum between 0.5 and 25 Hz showed that these age-dependent changes were most prominent in the SWA frequency range (Fig. 5.1.3).

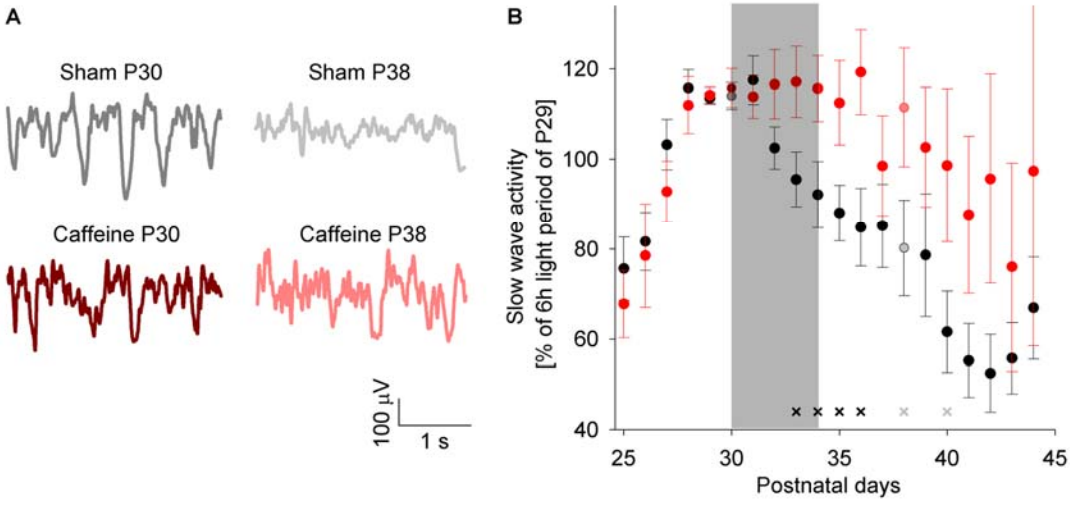

Figure 5.1.2: SWA trajectory across age in sham and caffeine treated animals. (**A**) Sample ECoG traces of a sham and caffeine treated animal on P30 and P38, respectively. **(B)** Trajectory of sleep slow wave activity (ECoG power between 1 and 4 Hz, averaged over the first 3 hours after light onset) between postnatal day 25 (P25) and P45 for sham (n=17) and caffeine (n=11) treated animals. The grey shaded background illustrates the period of caffeine administration. A two-way repeated measures ANOVA with factor age (P25-P45) and condition (caffeine and sham) was significant for age and condition ($p<0.05$). Crosses indicate increased SWA in caffeine compared to sham treated animals (black, $p<0.05$, gray, $p<0.08$), unpaired Student's t-test).

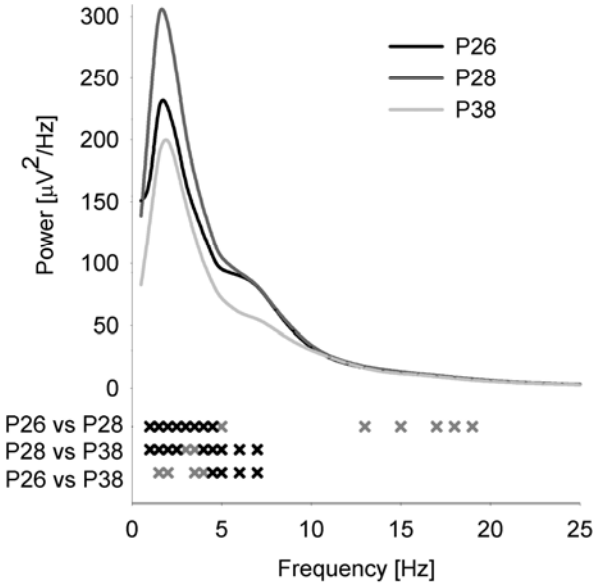

Figure 5.1.3: ECoG NREM sleep spectrum across age. Average NREM sleep ECoG power between 0.5 and 25 Hz across 24 hours for sham treated animals is shown for P26 (n=17), P28 (n=17) and P38 (n=15). Crosses indicate power differences across age (grey, $p<0.05$, black, $p<0.01$), paired Student's t-test).

The changes in SWA were paralleled by behavioral changes assessed in a repeated free exploration task (P28 and P42), during which more mature animals showed increased explorative behavior of a novel object (Fig. 5.1.4C). The other

behavioral parameters (grooming, quiet waking see Fig 5.1.4A, B and general exploring, data not shown) did not change across age.

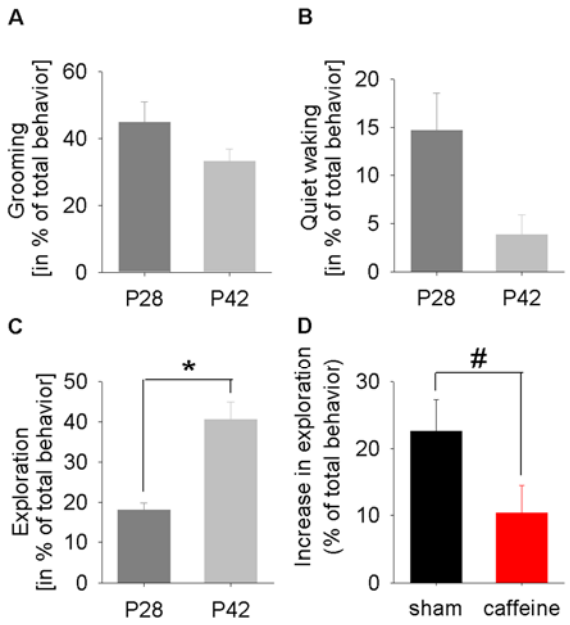

Figure 5.1.4: Behavioral changes across age. (A, B, C) The amount of grooming, quiet waking and exploration expressed as a percentage of total behavior are shown for P28 and P42. Significant changes across age are illustrated by an asterisk ($p<0.05$, paired Student's t-test). **(D)** The increase in object exploration time from P28 to P42 was reduced in caffeine (n=9) compared to sham (n=8) treated animals (# $p<0.05$, Mann-Whitney U-test).

Given the supposedly close relationship between SWA and cortical plasticity, we assessed structural changes in coronal sections stained for VAChT, a marker for cholinergic presynaptic terminals before and after the critical period when SWA decreased (P30 and P42). Representative sections are provided in Figure 5.1.6A. A quantification showed a significant reduction of the VAChT stained area expressed as percentage of total area at older age (Fig. 5.1.6B).

To manipulate sleep wake regulation during the critical period when SWA started to decline on P30 we administered caffeine via the drinking water for 5 consecutive days.

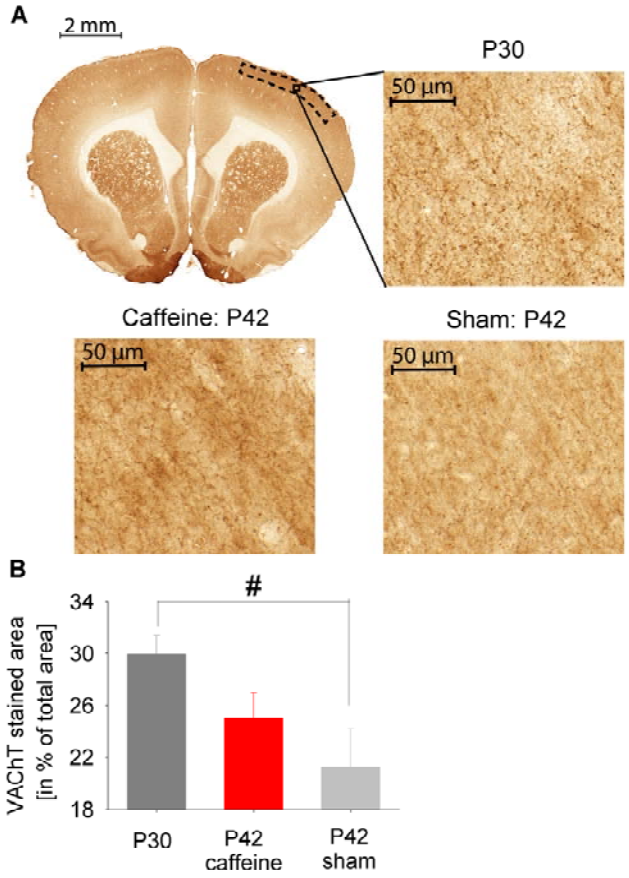

Figure 5.1.6: Structural changes across age. (A) Representative example image of a coronal section stained for vesicular acetylcholine transporter protein (VAChT), a specific marker for cholinergic presynaptic terminals. The dotted black box indicates the location of the randomly selected images for further analyses. One example, indicated by the solid black box is enlarged for postnatal day 30 (P30, right image). Below representative images for P42 after either sham or caffeine treatment are shown. (**B**) Reduction of the VAChT stained area, assumed to reflect cholinergic presynaptic terminals from P30 to P42 (n=6 per group, * $p<0.05$, Mann-Whitney U-test). Caffeine treated animals show a diminished reduction of presynaptic cholinergic terminals at P42.

Caffeine exerts short-term stimulating effects

Moderate caffeine intake (16.0 ± 1.4 mg/kg per day, see Materials and Methods for details) resulted in an expected increase in wakefulness and a reduction of NREM sleep during the dark period on the first day after the start of caffeine treatment (Fig. 5.1.1). No significant changes in vigilance states were observed in subsequent days (data not shown). Moreover, caffeine treatment led to a reduced build-up of slow wave energy (SWE), a measurement quantifying the accumulation of SWA across time and therefore controlling for the amount of wakefulness, during the initial two days of caffeine treatment (Fig. 5.1.7).

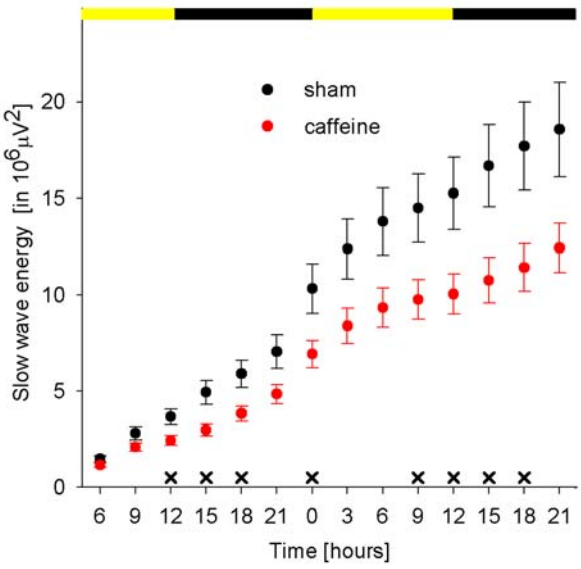

Figure 5.1.7: Caffeine reduces the build up of slow wave energy (SWE) during early caffeine treatment. Filled circles represent accumulated SWE (see Materials and Methods for details) in 3 hour intervals during the period when caffeine was initiated on P30 until the end of the second day of treatment (P31). The period of caffeine administration is illustrated by the grey shaded background. The white and black bars at the top of each graph indicate the 12 hour light and the 12 hour dark period, respectively. Crosses indicate reduced SWE in caffeine (n=11) compared to sham (n=17) treated animals ($p<0.05$, unpaired t-test). Error bars indicate SEM.

Long-term effects of caffeine

After the end of caffeine treatment no changes in the amount of wakefulness and NREM sleep between the two groups were found (P35, data not shown; P38, see Fig. 5.1.1). However, we found significant changes in the trajectory of SWA in caffeine treated animals beyond the treatment period. Caffeine treated animals exhibited higher SWA, averaged over the first 3 hours after light onset, between P33 and P36. Trend level differences were still observed 5 days after caffeine treatment on P40 (Fig. 5.1.2B). The higher level of SWA in caffeine treated animals was observed across the entire 24 hours 1 day after caffeine treatment on P35 (data not shown). Moreover, while an age-dependent increase in novel object exploration was found in both groups (for sham, see Fig. 5.1.4C), the increase in exploration time was reduced in caffeine treated animals compared to untreated animals (Fig. 5.1.4D). The immunohistochemical analysis under sham maturation showed a decrease in the VAChT stained area across age, a marker for cholinergic presynaptic terminals. After caffeine treatment this area was diminished (Fig. 5.1.6B).

DISCUSSION

This study used a combination of electrocortical (ECoG) recordings, behavioral and structural readouts to track maturational changes in the juvenile rat and their manipulation by caffeine. Our findings show that sleep slow wave activity (SWA) in the rat follows a similar inverted U-shaped trajectory as already known for humans (Campbell and Feinberg, 2009, Feinberg, 1982, Gaudreau et al., 2001, Jenni et al., 2004). Furthermore, mild caffeine treatment during the critical period when sleep SWA normally declines (between postnatal 30 (P30)-P34) affected sleep and resulted in alterations in maturational parameters. First, the SWA decline was delayed after caffeine treatment. Second, caffeine treatment reduced the normal increase in exploratory behavior. And last, the decrease of a marker for cholinergic presynaptic terminals across age was diminished in caffeine treated animals.

In humans it is well known that SWA during NREM sleep shows prominent age-dependent changes during the first two decades of life (Jenni and Carskadon, 2004, Feinberg and Campbell, 2010, Kurth et al., 2010, Gaudreau et al., 2001, Feinberg and Campbell, 2013). It was repeatedly shown that initially SWA increases, peaks shortly before puberty, and decreases during adolescence. Our results show a similar inverted U-shaped trajectory of SWA in the juvenile rat compared to humans. Moreover, peak SWA in humans is reached before puberty. Correspondingly, SWA in the rat peaks on P30, a period which was related to the beginning of puberty (Lee et al., 1975). Therefore, SWA in both species follows a similar trajectory during a comparable developmental period suggesting the rat to be a good model for further investigations. The age-dependent changes, though most pronounce, were not entirely restricted to the SWA frequency band but extended in the theta frequency range (6-7 Hz). This observation is in line with other studies reporting age-dependent changes which are not only restricted to the SWA frequency range (Feinberg and Campbell, 2010, Kurth et al., 2010). Moreover, sleep deprivation studies show that the prominent increased power in the SWA band extends into the theta frequency range (Finelli et al., 2000).

Due to the similar trajectory of synapse density and SWA, Campbell and colleagues suggested that the decrease of synapse density during adolescence is reflected in the decrease of SWA during this developmental period (Campbell and Feinberg, 2009). A mechanistic explanation for this close relationship is based on the observation that synchronization of cortical activity is a key factor determining the level of SWA (Vyazovskiy et al., 2009). Thus, increased synaptic density would enable faster synchronization of network activity, resulting in more SWA. Our microstructural data of the VAChT stained area provides a measure for presynaptic cholinergic terminals in the cortex (Gilmor et al., 1996). Moreover, it was shown that VAChT overexpression leads to increased acetylcholine (Ach) release and to an increased amplitude and frequency of miniature excitatory postsynaptic currents (Song et al., 1997). These results suggest a close relationship between VAChT and cholinergic signalling. Thus, the reduction of VAChT stained area seems to reflect a reduction in cholinergic signalling which parallels the reduction of SWA from P30 to P42. Together with evidence that the entire cerebral cortex matures as an integrated network (Lidow et al., 1991), our results support the proposed mechanistic

relationship between synaptic density and SWA in the rat. However, our interpretation is based on the assumption that VAChT also during cortical development provides a good measure for functional cholinergic synapses. This assumption is supported by a study in rats showing the highest Ach transmitter release, the best marker for functional synapses, on P30 (Pedata et al., 1982). This goes along with the proposed function of Ach during postnatal development providing a favourable environment for neuronal plasticity processes (Guizzetti et al., 2008, Lohmann et al., 2002, Zhu and Waite, 1998). Several studies provide additional evidence for structural changes to occur during the inverted U-shape trajectory of SWA. For example, an *in vitro* study in rats showed a similar inverted U-shaped trajectory of synapses per neuron peaking also around P30 (Ichikawa et al., 1993). Moreover, markers of postsynaptic density (PSD) show a similar developmental trajectory. For example, it was shown that postsynaptic protein 95 (PSD-95), a marker protein of postsynaptic density (Hunt et al., 1996) involved in maturation of excitatory synapses (El-Husseini et al., 2000), shows age-dependent changes. PSD-95 was massively increased at the beginning of the 4th postnatal week compared to postnatal week 1 and postnatal week 9 (Swulius et al.). Interestingly, PSD was found to be regulated in an activity-dependent manner (Ehlers, 2003).

We did not find evidence for a direct link between sleep variables, behavioral and structural changes. This is or might be due to the following reasons: Correlations between structural findings and sleep ECoG changes are not possible since the animals needed to be killed at the selected time points. In addition, the number of animals for which we have the combined measures (behavior and ECoG) is rather low making it difficult to find correlative evidence. A limitation, which has to be considered when looking at the trajectory of sleep SWA, is that data collection started immediately after surgical termination which did not allow the animal to undergo a recovery period. However, for our main findings, i.e. the decrease of SWA during the post-pubertal period (e.g after P30) and the diminished reduction of SWA after caffeine application, the animals had at least 5 days of recovery after surgery. Even tough we did not expect any post surgical effects on our main findings we cannot exclude any impact of the surgery on the prior increasing portion of the SWA trajectory (between P26 and P30). A repeated measure ANOVA with day of surgery as a between-subject factor (P23, P24 and P25) and SWA trajectory (age) as a

within-factor showed a significant effect of age. However, no interaction between age and day of surgery was found, providing good evidence that the recovery period did not affect the recording also during the early days (between P26 and P30). Moreover, we have conducted a control experiment during which the same surgical procedure followed by ECoG recordings was applied in animals at older age. The same analyses did not show an inverted U-shaped trajectory as observed in juvenile rats (data not shown).

Caffeine affected sleep in juvenile rats. In line with the literature, we found a similar short-term effect of caffeine on vigilance states. Initial caffeine treatment led to increased wakefulness and decreased NREM sleep (Schwierin et al., 1996). A caffeine induced increase in wakefulness was shown to be associated with an increase in SWA during subsequent sleep periods (Schwierin et al., 1996). In contrast, our rather small increase in wakefulness during the dark period did not result in more SWA at the beginning of the next light period (Fig, 5.1.2, P32). This discrepancy might be due to different aims of the caffeine administration. Schwierin and colleagues aimed to perform a pharmacological sleep deprivation for approximately 4 hours by injecting caffeine intraperitoneally at light onset. However our rationale was not to increase wakefulness and we therefore administered caffeine via the drinking water during a time window the animals are naturally awake. One established way to correct for even small changes in the amount of wakefulness, e.g. like during our early caffeine treatment, is to calculate SWE. We found that SWE was reduced in caffeine treated animals. This suggests that caffeine reduces the build up of sleep pressure during waking as already proposed previously (Landolt et al., 1995a, Schwierin et al., 1996).

One limitation of our approach was that caffeine administration was performed via the drinking water in order to not induce any stress, which was shown to directly influence SWA (Meerlo et al., 1997), one of our main parameters. The administration of caffeine via the drinking water provided a measure of overall daily caffeine consumption but did not allow further specification about the circadian time (between 6 PM - 2 AM) when caffeine was consumed.

Caffeine also led to long-term effects on the SWA trajectory. More precisely, we observed a delayed reduction of sleep SWA after caffeine application. Interestingly, we found similar results for our behavioral and structural marker of maturation. More

specifically, 7 days after the end of caffeine administration we found behavioral and structural changes: 1) Caffeine treatment led to a less mature behavior as assessed by object exploration, which increased as a function of age. 2) VAChT stained area, presumably reflecting mainly cholinergic presynaptic terminals, significantly decreased across age, while caffeine treatment led to a diminished reduction. In conclusion, these results propose that caffeine has long-lasting effects on maturation and the SWA trajectory closely parallels these changes. These parallel trajectories strengthen the observation that sleep SWA might be used as an electrophysiological marker of cortical maturation (Buchmann et al., 2011b, Kurth et al., 2012). That caffeine is able to interfere with the trajectory of brain morphological changes during development has been shown previously. For example, caffeine was shown to have a long-lasting effects on morphological parameters such as dendritic length in the neonatal rat in the prefrontal cortex (Juarez-Mendez et al., 2006) as well as in the middle age rat in the hippocampus preventing cognitive decline (Vila-Luna et al., 2011). Knockout models may help to explore the underlying mechanism of a potential role of adenosine signalling during cortical maturation. Several studies explore the effects of adenosine A_1, A_{2A}, A_{2B} or A_3 receptors in single knockout mice (Gimenez-Llort et al., 2002, Johansson et al., 2001). However, our applied caffeine dose mainly affects A_1 and A_{2A} receptors and no study investigates how cortical maturation is affected during a similar developmental period in A_1, A_{2A} receptors double knockout mice. Thus, a potential role of adenosine via adenosine receptors during cortical development has not been explored yet. Moreover, whether or not such caffeine-induced changes in morphology are due to alterations in sleep regulatory processes has not been investigated. Our data indicate that this might be the case. This interpretation is supported by the synaptic homeostasis hypothesis (Tononi and Cirelli, 2006) which proposes a key role of sleep slow waves in synaptic plasticity. Thus, altering sleep wake regulation by caffeine, reflected in changes of SWA, may affect synaptic plasticity. Nevertheless, there are alternative explanations of how caffeine may interfere with synaptic plasticity. For example, caffeine reduces the number of microglia (Steger et al., 2012) which seem to be important for synaptic pruning (Paolicelli et al., 2011). Or, caffeine blocks the activation of Cofilin (Rex et al., 2009), an actin binding protein, which is thought to be important for synaptic plasticity (Matus et al., 2000).

No matter what mechanism applies our study shows that caffeine interferes with cortical maturation during a critical developmental period. This might also be of clinical importance since the critical period of synapse elimination during adolescence is associated with an increasing incidence of psychiatric and mood disorders, such as schizophrenia, anxiety, substance abuse and personality disorders (Paus et al., 2008). Thus, it will be important for future studies to investigate the effects of chronic caffeine application during critical periods of development in animal models for psychiatric and mood disorders.

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5.2 Diurnal changes in EEG sleep slow wave activity during development in rats

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SUMMARY

According to the homeostatic regulation of sleep, sleep pressure accumulates during wakefulness, further increases after sleep deprivation and dissipates during subsequent sleep. Sleep pressure is electrophysiologically reflected by EEG slow wave activity (SWA) during NREM sleep and is thought to be stable across time.

During childhood and adolescence the brain undergoes massive reorganization processes. SWA during these developmental periods has been shown in humans to follow an inverted U-shaped trajectory which recently was replicated in rats. The goal of this study was to investigate in rats the diurnal changes of SWA during the inverted U-shaped developmental trajectory of SWA.

To do so, we performed longitudinal electrocorticogram recordings and compared the level of SWA at the beginning to the SWA level at the end of 24-hour baselines in two sets of Sprague-Dawley rats. In younger animals (n=17) we investigated specific postnatal days when overall SWA increases (postnatal day 26, P26), peaks (P28) and decreases (>P28). The same analysis was performed in older animals (P48, n=6). Our results show a gain of SWA across 24 hours on P26, followed by no net changes on P28, which was then followed by a loss of SWA during subsequent days (>P28). Older animals did not show any net changes in SWA across 24 hours. These results cannot be explained by differences in vigilance states. Thus, SWA during this developmental period may not only reflect the time course of sleep pressure but may additionally reflect maturational processes.

INTRODUCTION

According to the homeostatic regulation of sleep, sleep pressure accumulates during the time spent awake and dissipates during subsequent sleep (Borbely and Achermann, 2005). This homeostatic regulation of sleep pressure ensures that under constant baseline conditions no sleep pressure accumulates over time (days and weeks). A well established electrophysiological marker of the homeostatic changes in sleep pressure is slow-wave activity (SWA, EEG power between 0.5 and 4.5 Hz) during NREM sleep. It has been manifold shown that SWA accumulates during the waking period (Borbely and Achermann, 2005), further increases after sleep deprivation (Borbely and Achermann, 2005), and decreases during naps or nighttime sleep episodes (Borbely and Achermann, 2005). Taken together, in adults SWA precisely reflects the prior sleep/wake history and provides an accepted electrophysiological marker of the homeostatic regulation of sleep.

Adolescence is a translational period between puberty and adulthood during which many processes such as brain connectivity, cognitive skills and sleep/wake parameters undergo maturational changes. It is well established that SWA in humans shows during childhood and adolescence an inverted U-shaped trajectory which parallels the changes in measures of brain connectivity, e.g. synaptic density (Campbell and Feinberg, 2009). Recently, we were able to confirm a similar trajectory of SWA in rats during a comparable developmental period (Olini et al., 2013). Taken together, SWA in humans as well as in the rat follows a similar inverted U-shaped trajectory. During childhood (or pre-puberty in rats) SWA first increases, peaks before puberty (human: ~9 years, rat: ~postnatal day 28), which is then followed by a decline during subsequent years or days, respectively. In humans the period during which SWA undergoes dramatic changes requires several years, whereas in the rat this process develops within a few weeks. Therefore, the rat provides a good model which allows us to monitor markers of sleep pressure longitudinally for the period during which SWA undergoes massive maturational changes. The goal of this study was to investigate the diurnal changes in SWA in the rat before the developmental peak of SWA was reached, right on the peak and during subsequent days, when overall SWA significantly declines.

MATERIALS AND METHODS

All animal protocols followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and facilities and were approved by the Cantonal Veterinary Office of Zurich. Part of the present dataset has been analysed and is included in a previous publication (Olini et al., 2013) which mainly focuses on the trajectory of SWA during pre-puberty and puberty (P26-P45). For this analysis only the first 3 hours after light onset were analysed.

Surgical procedures

Animals were delivered after weaning with 22 (n=17) or 36 days of age (n=6). To immediately acclimatize the animals to the recording boxes all animals were placed in their individual box on the day of arrival, 3 days before the recording started. Then, we performed surgery according to a previously published protocol (Franken et al., 1991) in either 23- to 25-day or 37 to 39 days old male Sprague-Dawley rats, respectively. In this surgery, under isoflurane anesthesia all animals were implanted epidurally with gold-plated miniature screws (0.9 mm diameter) for electrocortical recording (ECoG) [right hemisphere: the frontal, 1.5 mm anterior to Bregma, 2 mm lateral to the midline and the parietal, 2 mm anterior to lambda, 2 mm lateral to midline; reference: above cerebellum, 2 mm posterior to lambda, on the midline]. Two gold wires (0.2 mm diameter) were inserted bilaterally into the neck muscles for electromyogram (EMG) recording. The electrodes were connected to stainless-steel wires and fixed to the skull with dental acrylic cement. All animals received a single dose of the analgesic Temgesic (0.1 mg/kg Buprenorphin, s.c.) during the last 30 min of the surgery. After the surgery no animal lost weight, instead all animals showed normal weight gain across the experiment. At the end of the experiment all brains were carefully inspected and no cortical damage was observed.

Electrocortical recordings

For the longitudinal recordings rats were connected by a fine cable to a swivel and remained connected throughout the experiment. Data collection started immediately after surgery for 20 consecutive days. Animals were singly housed and kept under a 12 hour light (9 AM to 9 PM) and 12 hour dark period. Food and water was given ad libitum. The ECoG and EMG signals were amplified (amplification factor, ~2000), filtered (highpass filter: -3 dB at 0.016 Hz; low-pass filter: -3 dB at 40 Hz) sampled with 512 Hz, digitally filtered [ECoG: low-pass finite impulse response (FIR) filter, 25 Hz; EMG: bandpass FIR filter, 20-50 Hz], and stored with a resolution of 128 Hz. The ECoG power spectra were computed for 4 s epochs by a fast Fourier transform routine. Adjacent 0.25 Hz bins were averaged into 0.5 Hz (0.25-5 Hz) and 1.0 Hz (5.25-25 Hz) bins. Before each recording, the EEG and EMG channels were calibrated with a 10 Hz, 300 μV peak-to-peak sine wave.

Data analyses

Vigilance states (NREM sleep, REM sleep and Wake) were visually determined, as previously documented (Franken et al., 1991), by off-line visual inspection of the ECoG and EMG signals. We selected 24 hours recording of representative days throughout the longitudinal recordings: postnatal day 26 (P26), P28, P31, P36 and P38 in animals who underwent surgery between P23-P25 and P48 in animals who underwent surgery between P37-P39. Due to technical constrains the 12 hour light or dark period did not always contain a total recording time of 12 hours. Only animals with a minimum of 10 out of 12 hours recording time were included in our analyses of vigilance states. As a result for the light period only 2 animals contributed to the entire data set.

Our analysis was restricted to the frontal derivation. All epochs containing artifacts in one derivation were excluded from spectral analysis. Due to bad signal quality of the frontal derivation one animal had to be excluded. The time course of SWA across 24 hours was assessed by three representative 40-min NREM sleep intervals: the first interval at the beginning of the light period, a second interval at the beginning of the dark period and the third interval at the end of the dark period. A 40-min time interval was chosen as a trade off between a robust measure and an as

short as possible time window during which the 40 min of NREM sleep were distributed. This trade off was of particular importance at the end of the dark period due to a limited amount of sleep occurring at that time. Data analyses and statistics were performed using the MATLAB software package (MathWorks). Contrasts were tested by post-hoc parametric paired and unpaired Student's t-tests after significance in a one way or two way ANOVA with repeated measures, with the exception of vigilance states during the light period for which missing data (see above) prevented a repeated measures ANOVA.

RESULTS

For the assessment of the homeostatic regulation of sleep during development, we performed longitudinal electrocortical recordings (ECoG) in male Sprague-Dawley rats across 24 hours between postnatal day 25 (P25) and P42, the age range when SWA follows an inverted U-shaped trajectory in juvenile rats. When looking at 1-h intervals across 24 hours, the overall time course of SWA looks similar for all assessed postnatal days (Fig. 5.2.1A, in grey). At light onset SWA reaches a maximum followed by a reduction in SWA across the light period, the rat's main sleeping period. Then during the dark period SWA continuously increases. We then investigated the average time course of SWA across all animals by calculating SWA for the first 40 min after light onset, the first 40 min after dark onset and the last 40 min of NREM sleep during the dark period (see Methods) for P26, P28, P31 and P48 (Fig. 5.2.1A, in black). Also this analysis showed that all animals exhibited a prominent SWA decline across their main sleeping period indicated by a significant decline in SWA from the first interval of the light period to the first interval of the dark period (Fig. 5.2.1A).

In a next step we assessed net changes across 24 hours by comparing the first and the last 40 min of NREM sleep. When animals displayed peak SWA on P28, we found no difference of SWA between the first and the last 40 min of NREM sleep (Fig. 5.2.1B). However, before peak SWA was reached (<P28, Fig. 5.2.1B), we found a gain of SWA across 24 hours, reflected by significantly higher SWA values in the last compared to the first 40 min of NREM sleep. During the postnatal days when

overall SWA declines (>P28, Fig. 5.2.1B), SWA was lost across a day - now the last 40 min showed lower SWA compared to the first 40 min of NREM sleep. In a different group of older animals (P48) we found a significant reduction of SWA between the first 40 min of NREM sleep after light onset and the first 40 min of NREM sleep after dark onset. As expected for an adult animal, the SWA comparison between the first and last 40 min interval revealed no difference in P48 old animals (Fig. 5.2.1B).

To assess whether a redistribution of vigilance states across the 24 hours may account for the observed age-dependent changes in the SWA time course we analysed the changes in vigilance states during the light and the dark period. In general, as expected at that age the animals showed clear light/dark changes in all vigilance states with a NREM sleep predominance during the light period and wake predominance during the dark period (Fig. 5.2.2 and 5.2.3). In general, animals at all ages showed a similar distribution of the vigilance states across 24 hours recording time (Fig. 5.2.2). Furthermore, we found that during the dark period the amount of wakefulness increases with age (Fig. 5.2.3). This age-dependent increase in wakefulness is compensated by a reduction of NREM and REM sleep.

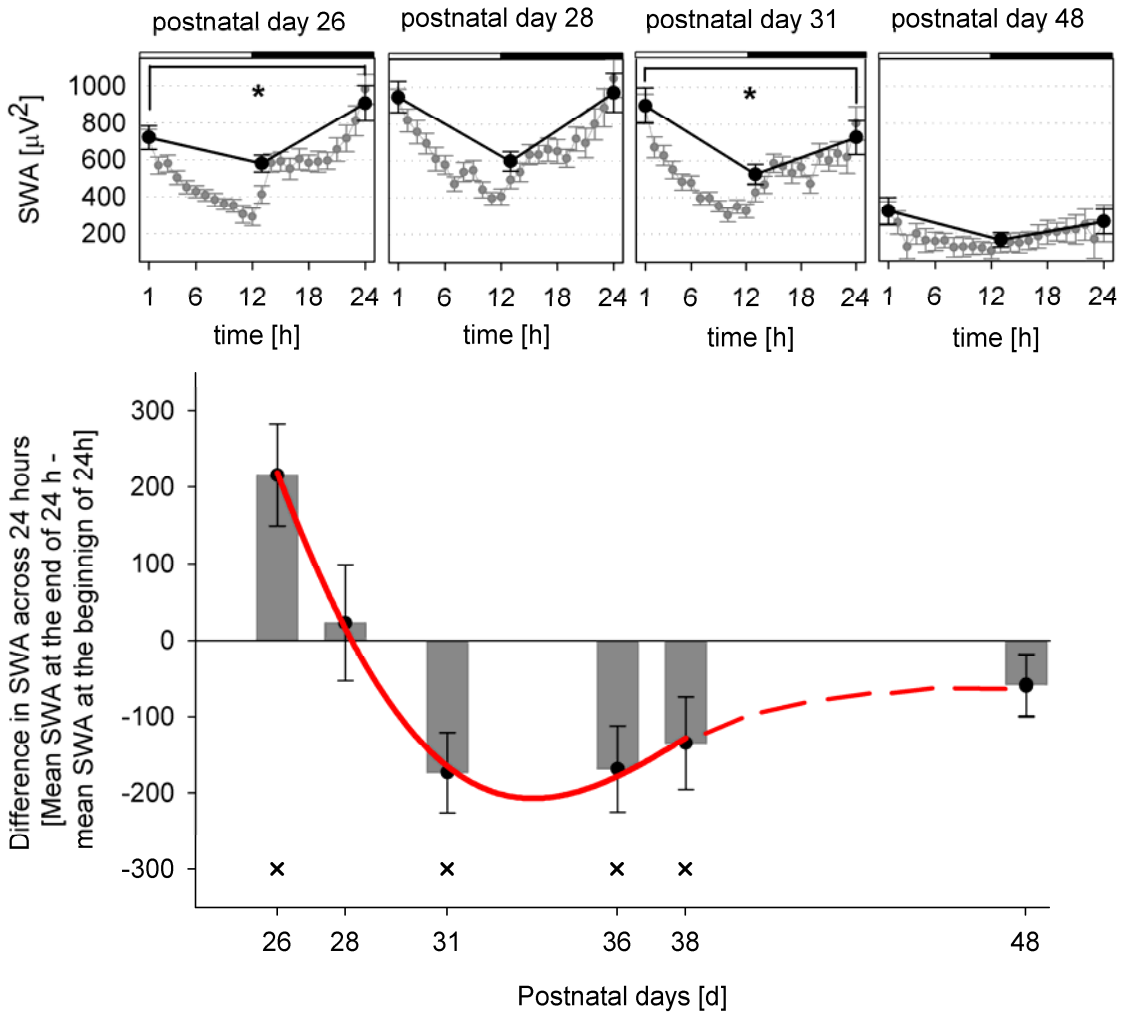

Figure 5.2.1: Daily changes in slow wave activity across age. **A)** In grey, mean SWA is shown in 1-h intervals for P26, P28, P31 and P38. In black, mean SWA is shown for the first 40 min of NREM sleep during the light period, the first 40 min of NREM sleep of the dark period and the last 40 min of NREM sleep of the dark period. The yellow and dark bars illustrate the12 hours light and 12 hours dark period, respectively. ANOVA with repeated measures for age: P26 (n=16), P28 (n=17), P31 (n=17), P36 (n=16) and P38 (n=15) and interval (first 40 min interval of the light period, first 40 min interval of the dark period and last 40 min interval of the dark period) was significant for age, interval and its interaction. Asterisk's indicate

significant differences between first and last 40 min interval for P26, P28, P31 and P38 (paired Student t-tests, $p<0.05$). In addition, SWA between the first interval of the light period and the first interval of the dark period showed a significant decline across all days (paired Student t-tests, $p<0.05$). This decline in SWA was followed by a significant increase in SWA between the first and the last interval of the dark period for all days except for P48 (paired Student t-tests, $p<0.05$). **B)** The graph shows the difference of mean slow wave activity (SWA) of the last 40 min of NREM sleep of the dark period and the first 40 min of NREM sleep of the previous light period after significance in Anova with repeated measures for age: P26 (n=16), P28 (n=17), P31 (n=17), P36 (n=16) and P38 (n=15). Crosses indicate the difference of SWA compared to no change (0 line, unpaired Student's t-test, $p<0.05$; P48: n=6). Error bars indicate SEM.

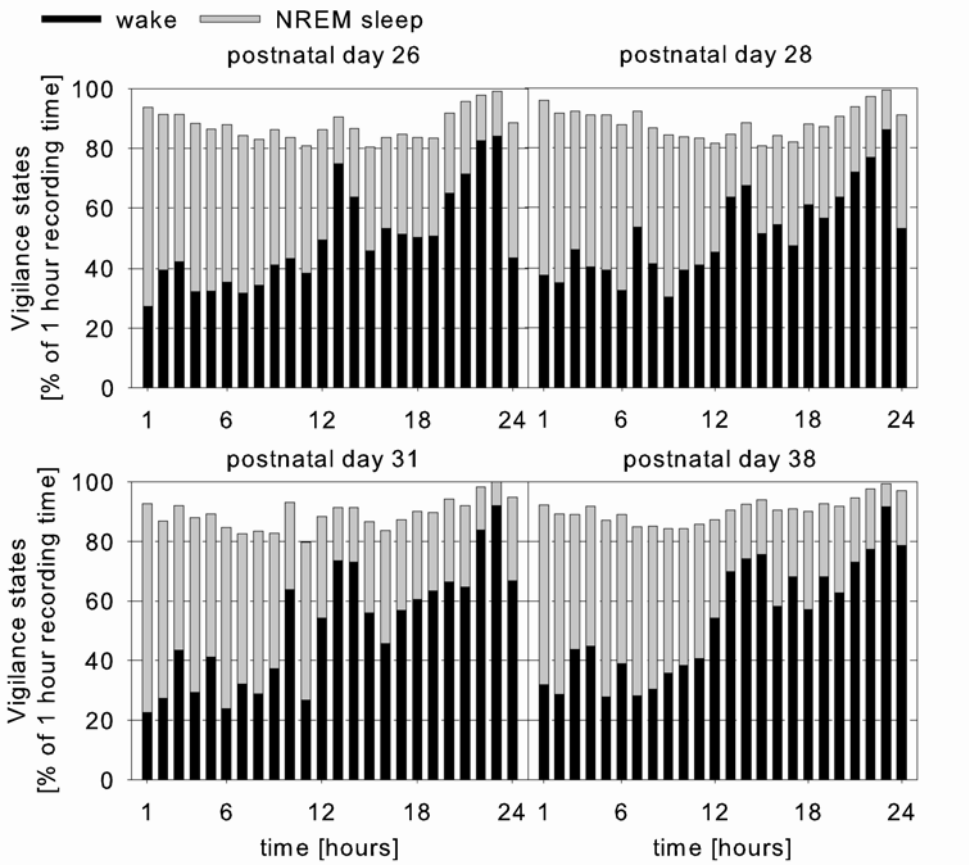

Figure 5.2.2: Vigilance states across age. Wake and NREM sleep are expressed as a percentage of 1-h recording time across one day for P26, P28, P31 and P38.

Interval 1 to 12 and 13 to 24 correspond to the 12 hours light and 12 hour dark period, respectively. For statistical analyses see Figure 5.2.3.

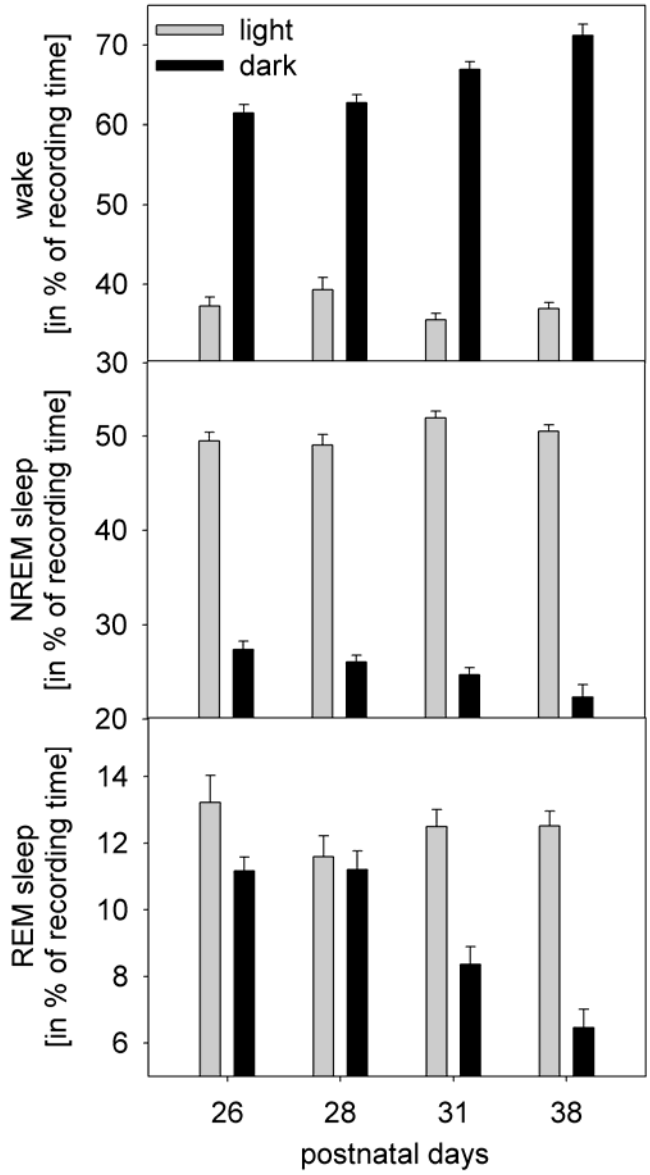

Figure 5.2.3: Visually scored vigilance states across age. Wake, NREM and REM sleep is shown for the light period in grey and the dark period in black for postnatal day 26 (P26), P28, P31 and P38 in percent of recording time. One way ANOVA with repeated measures showed a significant age factor for the dark period for all

vigilance states (with P26 (n=17), P28 (n=17), P31 (n=17) and P38 (n=16)). Error bars indicate SEM.

DISCUSSION

This study longitudinally followed the homeostatic regulation of sleep, as reflected by the diurnal changes of ECoG SWA during a time period when SWA undergoes massive changes in humans as well as in rats (Feinberg, 1982, Gaudreau et al., 2001, Jenni et al., 2004, Campbell and Feinberg, 2009, Olini et al., 2013). Both rats and humans show a similar inverted U-shape trajectory of SWA during development. During this developmental period, the diurnal changes of SWA follow a typical pattern at all ages: SWA decreases during the light period during which the rats are predominantly asleep and increases during the following dark period when rats are predominantly awake. Thus, as expected based on the homeostatic regulation of sleep, sleep pressure, as reflected by SWA, dissipates during sleep and accumulates during wakefulness at all assessed ages. However, when looking at net changes across 24 hours we found significant age-related differences. At P26 the SWA decrease during the light period was smaller than the increase during the dark period. At P31, P36 and P38 we found the opposite, now the SWA decrease during the light period was larger than the increase during the dark period. As a consequence, rats at an age before peak SWA was reached (P26) gain and rats at an age after peak SWA (P31, P36, P38) loose SWA across 24 hours. Rats exhibiting peak SWA (P28) do not show such net changes.

According to the two-process model sleep is regulated by two independent processes, a circadian process C and a homeostatic process S (Borbely, 1982). As an electrophysiological marker of sleep homeostasis SWA depends on prior sleep-wake history (Borbely and Achermann, 2005). Thus, age-dependent changes in the distribution of vigilance states may account for the observed differences. However, wakefulness during the 12 hours dark period continuously increases during development which did not lead to a progressive increase of SWA at the end of the dark period. Moreover, we found no obvious re-distribution of vigilance across the 24 hours.

Although process S and C are assumed to be independent a shift in the circadian rhythm as observed during a similar age span in human development (Carskadon et al., 1993) may contribute to our observed net changes in SWA. However, we did not find any evidence for such a circadian shift in our rats when looking at the 1 hour vigilance states distribution across 24 hours. At all ages rats were well adapted to the 12-h light dark period. Recent studies indicate that process S and C might not be completely independent from each other (Curie et al., 2013). Furthermore, a study in zebrafish larvae focusing on the dynamics of synaptic modifications observed circadian rhythmicity in synapse number in hypocretin/orexin neurons (Appelbaum et al., 2010). These studies show that circadian rhythms may affect SWA and measures of synaptic connectivity. Circadian rhythmicity investigated, for example, by the expression of clock genes in the SCN was shown to mature during the first 3 postnatal days in rats (Kovacikova et al., 2006). However, there are no studies investigating how these clock genes may subsequently change and thus we cannot exclude a potential circadian influence on our results.

Another limitation which has to be considered when interpreting the inverse change of SWA across 24 hours is that data acquisition started immediately after surgery which did not allow the animals to undergo an appropriate recovery period of about 5 days. However when animals showed a net loss of SWA (>P30) or balanced SWA across 24 hours in older animals (P48), the animals had at least 5 days postsurgical recovery. Even tough we did not expect any effects of the surgery on these findings, we can not rule out an impact of the surgery on the earlier data points (P26 and P28). A repeated measure ANOVA with day of surgery as a between-subject factor (P23, P24 and P25) and the net change of SWA across 24 hours at different age (P26, P28, P31, P36 and P38) as a within-factor showed a significant effect of age. However, no interaction between age and day of surgery was found, providing good evidence that the recovery period did not affect the recording also during the early days (P26 and P28).

The two-process model of sleep regulation proposes that SWA closely reflects the homeostatic regulation of sleep pressure. The model predicts a balance of sleep pressure across days during adulthood. Interestingly, our results show that during development in rats the major electrophysiological marker of sleep pressure is not always balanced. Few studies have investigated the development of the homeostatic

regulation of sleep. Although SWA can be quantified immediately after birth, SWA during this early period seems not to reflect the homeostatic regulation of sleep. A study in young rats reported a diurnal organization of vigilance states between P16 and P20, however, the adult-like decline of SWA across the light period was not observed before P24 (Frank and Heller, 1997). This lack of a decline in SWA indicates that sleep homeostasis is not developed before P24, which was supported by a study applying sleep deprivation during a similar age span. In this study, before P24, sleep deprivation led primarily to a compensatory increase in NREM sleep (Frank et al., 1998). However, sleep deprivation in juvenile rats at P24 resulted in the adult-like increase in SWA during recovery sleep (Frank et al., 1998). A more recent study investigated the effects of sleep deprivation in mice from P19 to adulthood. They found a consistent increase in SWA during recovery sleep only after P42 (Nelson et al., 2013). Nevertheless, during baseline the authors found the same homeostatic SWA changes from adolescence to adulthood, starting from P19: SWA declined in the course of sleep and increased across periods of spontaneous wake. The authors hypothesized that the inconsistent increase in SWA after sleep deprivation in adolescent mice may derive from a ceiling effect. Taken together, several studies showed a mature homeostatic regulation of SWA in rats during the age span of our study. Thus, once sleep homeostasis is fully developed the observation that net SWA changes across 24 hours raises the question whether SWA during this age span not always reflects the level of sleep pressure. The relationship between SWA and cortical connectivity (strength or density) may provide an explanation for these net SWA changes. Multiunit recordings in the rat show that synchronization of cortical activity is a key factor determining the level of SWA (Vyazovskiy et al., 2009). In turn, synchronization is tightly related to cortical connectivity (Whitlock et al., 2006). This indirect link between SWA and cortical connectivity is of relevance for our study because it is well known that cortical connectivity shows prominent changes during development in humans and non-human primates (Huttenlocher, 1979, Huttenlocher and Dabholkar, 1997, Rakic et al., 1986) as well as in the rat (Nakamura et al., 1999). Although, synaptic formation and elimination are thought to happen concurrently (Yang et al., 2009), these studies show that during early development more synapses are formed than eliminated which is followed by a reversed process, when more synapses are eliminated than

formed. As a result of these net changes in synapses, overall cortical connectivity increases during early development and decreases thereafter before reaching stability during adulthood. This is in line with a two-photon imaging study showing that dendritic spines, precursor of synapses, remain plastic during development and become remarkably stable throughout the cortex (Grutzendler et al., 2002, Zuo et al., 2005). In summary, the age-dependent changes in cortical connectivity follow a similar inverted U-shape trajectory as sleep SWA does. More specifically, the increase in cortical connectivity may be reflected in the net increase of SWA during early development. During the adolescent period the decrease of cortical connectivity would then result in the observed net reduction of SWA. Thus, SWA during pre- and post-puberty not only reflects sleep pressure but seems to additionally reflect cortical maturational processes.

A remaining question is whether SWA actively contributes to these maturational processes. The synaptic homeostasis hypothesis proposes that SWA is responsible for synaptic downscaling, a process by which the learning-dependent increase in synaptic strength during wakefulness is re-normalized during subsequent sleep (Tononi and Cirelli, 2006). This regulation of synaptic strength is assumed to be in balance in adult organisms and is thought to be reflected by SWA (Tononi and Cirelli, 2006). In line with this, a study performed in adult mice showed that molecular and electrophysiological measures of synaptic strength were high after prolonged wakefulness and low after a consolidated sleep period (Vyazovskiy et al., 2008). Moreover, a two-photon study, performed in adolescent mice, found that sleep was associated with a net spine loss resulting in a reduction of synaptic connectivity, whereas wakefulness led to a net increase in cortical spines or synaptic connectivity (Maret et al., 2011). Such behavioral state-dependent changes in spines were however not found in adult mice. Thus, applying such methodology reveals sleep and wakefulness-dependent modulations of synaptic connectivity only during a developmental period with heightened synaptic plasticity, i.e. synaptogenesis during initial days followed by a period of synapse elimination or pruning (De Felipe et al., 1997, Yu and Zuo, 2011).

It might be of interest to speculate that the above mentioned regulation of synaptic strength is not balanced during development as was found for SWA. We proposed in a recent hypothesis (Ringli and Huber, 2011) that during early

development wakefulness leads to a higher synaptic connectivity than subsequent sleep is able to rebalance. Across time, this leads to an overall increase in synaptic connectivity. Then during the adolescence period the opposite picture is proposed, synaptic downscaling predominates over synaptic strengthening resulting in a decrease of synaptic connectivity. However, the study by Maret et al. found no evidence that spine loss or gain across sleep and waking changes as a function of age during early to late adolescence. Maybe other techniques being able to measure functional synapses are needed to detect the imbalance of synaptic strength across a day. One way to do so might be based on the experimental design by Vyazovskiy et al. (Vyazovskiy et al., 2008) continuously recording EEG (to assess SWA) and local field potential (LFP) responses evoked by electrical stimulation (as a marker of synaptic strength). In this way a direct relationship between age-dependent changes in synaptic strength and SWA becomes possible. Moreover, molecular markers associated with synaptic strength like the expression of AMPAR or levels of synaptoneurosomes could be investigated across age.

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5.3 Sleep homeostasis in a mouse model of depression

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ABSTRACT

Study Objectives: Depressive disorder in humans is closely related to sleep disturbances with insomnia as one of its symptoms. Sleep is known to be homeostatically regulated, which is electrophysiologically reflected by sleep EEG slow wave activity (SWA) during NREM sleep. It was hypothesized that the homeostatic regulation of sleep is disturbed in depression. Thus, the aim of the present study was to investigate sleep homeostasis by means of SWA in a recently developed mouse model of depression.

Design: Electrocortical recordings (ECoG) across 24-h baseline followed by a 4-h sleep deprivation and a 20-h recovery period.

Setting: Preclinical laboratory for translational research into affective disorder and rodent sleep research laboratory.

Patients or Participants: Twelve littermate trios of adult male C57BL/6 mice

Interventions: A subgroup of mice (CSD mice) underwent a 10-day chronic social defeat manipulation (CSD) that induces depression-relevant behavior.

Measurements and Results: After sleep deprivation, CSD mice showed a blunted increase in SWA compared to control (CON) mice. Vigilance states did not differ during baseline, sleep deprivation, and recovery in CSD mice compared to CON, with the exception of CSD mice exhibiting less REM sleep during the baseline dark period.

Conclusion: Our data provide evidence for a disturbed homeostatic regulation of sleep in CSD mice reflected by a slower build up of SWA during prolonged waking.

INTRODUCTION

Major depressive disorder (MDD) is one of the most potent risk factors for suicidality (Lewinsohn et al., 1994), and will be the second leading cause of burden of disease by 2030 (Mathers and Loncar, 2006). Sleep disturbances are closely related to MDD (Nutt et al., 2008, van Mill et al., 2010) with insomnia or hypersomnia listed as one of its symptoms (DSM-5, 2013 , ICD-10, 1994). Accordingly, several studies have reported altered sleep architecture in MDD patients compared to healthy controls. The most consistent changes in sleep architecture in depressive patients include shorter REM sleep latencies combined with increased total REM sleep and REM density, and diminished slow wave sleep (Palagini and Rosenlicht, 2011). Most studies performed to date have rather investigated changes in sleep architecture, whereas only little is known about altered sleep regulation in MDD (see below).

Sleep is known to be homeostatically regulated. Electrophysiologically this homeostatic regulation of sleep is reflected by sleep slow wave activity (SWA, EEG power, 1-4.5 Hz) (Borbely, 1982). Accordingly, SWA increases during wakefulness, further increases during sleep deprivation (Borbely et al., 1984a, Franken et al., 2001, Vyazovskiy et al., 2007, Webb and Agnew, 1971) and declines during subsequent naps or night-time sleep (Karacan et al., 1970, Werth et al., 1996). The homeostatic regulation of sleep in MDD has been shown to be disturbed, supported by studies showing reduced SWA during NREM sleep (Plante et al., 2012, Hoffmann et al., 2000, Borbely et al., 1984b). The S-deficiency hypothesis of Borbély et al. postulates altered sleep regulation in MDD (Borbely, 1987). More precisely, it proposes a slower build up of SWA during wakefulness in depressive subjects resulting in less SWA at sleep onset which is in line with the previously mentioned studies (Plante et al., 2012, Hoffmann et al., 2000, Borbely et al., 1984b). The S-deficiency hypothesis further provides an explanation for the beneficial effect of sleep deprivation in depressive patients (Fahndrich, 1981, Schilgen and Tolle, 1980, Giedke et al., 2003) by increasing their sleep pressure to a normal level.

More recently, a body of evidence suggests SWA to reflect cortical plasticity (Hanlon et al., 2009, Huber et al., 2006, Vyazovskiy et al., 2008). For example, SWA has been found to be locally increased after a visuomotor learning task. This local increase in SWA was restricted to the right parietal lobe, a region known to be involved in processing sensory information relevant for learning the corresponding task (Huber et al., 2004). The local increase in SWA was related to local synaptic potentiation mechanisms induced during learning the task. The opposite effect was found after reducing somatosensory input by a longer period of arm immobilization which was related to synaptic depression (Huber et al., 2006). Moreover, structural and functional brain plasticity impairments have also been associated with the pathophysiology of major depression (Duman et al., 2000, Krishnan and Nestler, 2008, Manji and Duman, 2001, Pittenger and Duman, 2008).

The aim of this study was to investigate the relationship between sleep regulation and depression in a recently developed mouse model for depression (Azzinnari et al., submitted). The mice underwent a 10-day procedure of chronic social defeat (CSD) comprising brief daily physical attacks by and otherwise continuous sensory exposure to dominant mice. Thereafter, electrocortical recording was conducted to assess effects on vigilance states and SWA in CSD mice relative to control mice during a 24 hour baseline recording, 4 hour sleep deprivation and the following 20 hour recovery period.

MATERIALS AND METHODS

Animals and maintenance

C57BL/6J mice were bred in-house. Male offspring were weaned at age of 3 weeks and caged in groups of three. In total, 12 littermate trios were used for this study. Mice were aged 9-14 weeks at study onset and weighed 24.0-34.0 g. Male CD-1 mice (Janvier, France, www.janvier-europe.com) were aged 8 months, ex-breeders, and caged singly at study onset. Mice were maintained on a reversed 12:12 h light-dark cycle (lights off 07:00-19:00 h) in an individually-ventilated caging

system (IVC) at 20-22 °C and 50-60% humidity. Cages were type 2L and contained woodchips, a sleep igloo and tissue bedding. Complete-pellet diet (Provimi, Kliba Ltd, Kaiseraugst, Switzerland) and water were available continuously and *ad libitum*. The study was conducted under a permit (110/2009) for animal experimentation issued by the Veterinary Office, Zurich, Switzerland. All efforts were made to minimize the number of mice used.

Study design

The study design is described in Figure 5.3.1. Each cage contained a littermate trio of C57BL/6J mice. Electrocortical surgery was performed with two mice per cage; the non-operated mouse remained in the cage and was allocated to the naive (Nv) group. Following post-surgery recovery, all mice were given a motor activity test in a fully automated apparatus (Multi Conditioning System, TSE Systems GmbH, Bad-Homburg, Germany) with light-beam sensors to detect movement, as previously described (Pryce et al., 2012). Briefly, mice were placed in a 30 x 30 cm arena on a floor consisting of stainless steel rods. The motor activity test was for 15 min and horizontal-vertical activity and freezing were measured. Activity was measured in arbitrary units and freezing behaviour was defined as the complete absence of activity for periods of at least 2 sec and expressed as % time freezing. The two operated mice per cage were allocated to either the chronic social defeat (CSD mice) or the control (CON) group such that the two groups were counter-balanced with respect to % time freezing in the motor activity test (t = -1.124 df = 20, p = 0.274). On the following day, mice allocated to CSD were transferred to the cage of a CD-1 mouse and CSD was performed for 10 days (see below). Naïve (Nv) and CON mice remained as pairs. On the day after CSD 10, mice were given a fear conditioning test as a behavioural measure of CSD efficacy. On the following day, mice were transferred to the ECoG laboratory and ECoG was performed for 2 consecutive days with CSD mice and CON mice. The first day served as baseline, which was followed by a 4-h sleep deprivation and a 20-h recovery period.

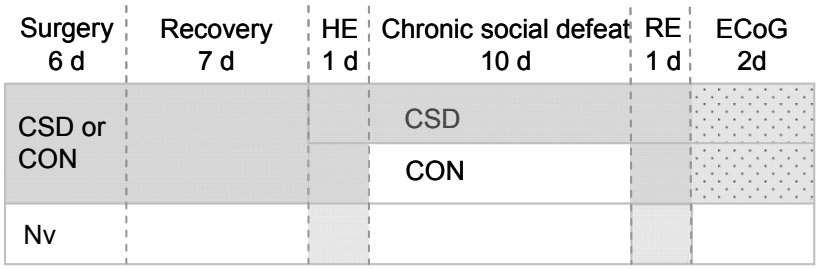

Figure 5.3.1: Overview of the study design. If a group is involved in specific steps of the experiment it is marked by a grey shaded background. The dotted area indicates the experimental part which was performed in the sleep laboratory. Motor activity test (HE), Fear conditioning readout (RE), electrocortical recording (ECoG).

ECoG surgical procedure

Surgery for electrocorticogram (ECoG) recording was conducted according to the protocol of (Franken et al., 1991). Under sevoflurane, mice were implanted on the dura with gold-plated miniature screws (∅ = 0.9 mm) using the following coordinates: right hemisphere, frontal, 1.5 mm anterior to bregma, 2 mm lateral to midline; parietal, 2 mm anterior to lambda, 2 mm lateral to midline; reference, above cerebellum, 2 mm posterior to lambda, on the midline. Two gold wires ((∅ = 0.2 mm) were inserted bilaterally into the neck muscles for electromyogram (EMG) recording. Electrodes were connected to stainless-steel wires that were in turn connected to a plug which was fixed to the skull with dental acrylic cement. Mice received one pre- and 2 post-operative doses of analgesic (0.1 mg/kg Buprenorphin, s.c.). Mice regained pre-surgery weight within 2-3 days and were given a 7-day recovery period prior to the next experimental procedure (habituation experiment (HE), Fig. 5.3.1). At the end of the experiment all brains were inspected carefully and observed to be free of cortical damage.

Chronic social defeat

Chronic social defeat comprised a mouse being placed in the cage of an aggressive CD-1 mouse on CSD day 1 (CSD 1) so that bouts of attack occurred. The mice remained together for a maximum of 60 sec of attack or for a maximum of 10 min. Thereafter the mice were separated by a central Plexiglas divider that was transparent and perforated to allow sensory communication but prevent physical attacks. After 24 h, the CSD mice were rotated between CD-1 mice cages and therefore confronted each day with a novel CD-1 aggressor. This procedure was repeated across 10 consecutive days. Daily attack durations were 30-60 sec. Bite wounds were prevented by trimming the incisor teeth of the CD-1 mice every third day using rodent tooth-cutting forceps. The detailed CSD protocol is given in Azzinnari et al. (Azzinnari et al., submitted). Two refinements of the protocol were made: the CSD duration was reduced from 15 to 10 days, to take into account that mice had also undergone surgery stress and to reduce the risk of ECoG-cap technical damage/failure; the central divider was removed during CSD to reduce the risk of head injury due to the ECoG cap. Naive and CON mice were handled daily. All mice were weighed daily.

CSD efficacy readouts

Two readouts were taken to assess the efficacy of CSD when combined with a surgical intervention and the chronic presence of the ECoG cap. In principle it was possible that CSD effects could be increased relative to CON due to additive or synergistic effects of the different stressors or decreased due to high effects of surgery-ECoG cap stress relative to CSD.

Daily body weight delta. Chronic social defeat induces a consistent increase in absolute day-to-day change in body weight, from about 1% to about 2% (Azzinnari et al., submitted). Percent day-to-day body weight delta (ΔBW) for 5-day time blocks of pre-CSD (day -3 – 1), CSD block 1 (day 2 – 6) and CSD block 2 (day 7 – 10) was calculated as: (abs(BW day n – BW day n-1)) /(BW day n-1)*100.

Fear conditioning. Chronic social defeat induces a robust increase in acquisition of contextual fear of electroshock, as measured in terms of freezing during the intervals

between shocks, which is about 20% and 40% time spent freezing in CON and CSD mice, respectively (Azzinnari et al., submitted). One day after CSD 10, contextual fear conditioning (Fig. 5.3.1, read out experiment) was conducted using the same apparatus as for the motor activity test, with all mice. Following a 5 min habituation, mice were exposed to 15 electroshocks of 3 sec x 0.15 mA with intervals of 50-sec (inter-trial intervals, ITIs). The major readout was mean % time spent freezing during ITIs.

Electrocortical recordings

For electrocortical recordings CSD and CON mice were transported to the sleep laboratory. There, CSD mice were separated by a divider from CD-1 mice, and CON mice were also separated by a divider from Nv. The ECoG cap was attached to a connector plug soldered to a fine cable and attached to a swivel. Mice were maintained under the same light-dark cycle (light on 19:00-07:00 h) as used throughout the experiment. Food and water was available *ad libitum*. Before each recording, the EEG and EMG channels were calibrated with a 10 Hz, 300 μV peak-to-peak sine wave. The ECoG and EMG signals were amplified (amplification factor ~2000), filtered (high-pass filter: -3 dB at 0.016 Hz; low-pass filter: -3 dB at 40 Hz), sampled with 512 Hz, digitally filtered (ECoG: low-pass finite impulse response (FIR filter, 25 Hz; EMG: band-pass FIR filter, 20-50 Hz), and stored with a resolution of 128 Hz. The ECoG power spectra were computed for 4 s epochs by a fast Fourier transform routine. Adjacent 0.25 Hz bins were averaged into 0.5 Hz (0.25-5 Hz) and 1.0 Hz (5.25-25 Hz) bins.

The vigilance states NREM sleep, REM sleep and Wake were determined visually off-line from the ECoG and EMG signals, as described in (Franken et al., 1991). Epochs containing artefacts in one derivation were excluded from spectral analysis of both ECoG derivations.

Sleep deprivation

Sleep deprivation (SD) was performed for 4-h, starting at light onset, according to (Franken et al., 1991). Briefly, mice were kept awake by placing new objects into the cage. Mice were video recorded during SD (Roline, RIC-45 IP Camera). Offline, on-going behaviours were scored from an ethogram using instantaneous sampling with a 10-sec interval; the mutually-exclusive behaviours scored were no behavior, quietly awake, grooming, exploring, object exploring and interaction at the divider between CSD mice or CON with CD-1 and Nv, respectively. In addition, all sensory stimulations by the experimenter to maintain mice awake i.e. disturb, object in, object out, were counted. In addition, the number of rearings, an additional measure for exploratory behaviour, was scored (Crusio, 2001).

Statistical analysis

ECoG data analysis and statistics were performed using the MATLAB software package (MathWorks). Analysis of variance was conducted with a between-subject factor of group (CSD, CON) and a within-subject factor of time period (2-h blocks), for the light and dark period separately. For ECoG power spectrum differences multiple unpaired Student t-tests were used. CSD efficacy measures were analysed by ANOVA using SPSS (version 20, SPSS Inc., Chicago IL, USA).

RESULTS

The mean daily duration of CSD attack was 44.5 $\pm$ 1.9 sec per mouse, which is typical for this CSD protocol. For absolute BW there was a significant group x time block interaction ($F(4, 58) = 3.140$, $p < 0.021$, Fig. 5.3.2A) but no significant effect of group at any specific time block ($p = 0.114$). For the CSD efficacy measure of % day-to-day ΔBW there was significant group x time block interaction ($F(4, 58) = 9.911$, $p < 0.0005$, Fig. 5.3.2B): % day-to-day ΔBW was increased in CSD mice relative to CON and Nv mice at CSD block 2. For the CSD efficacy measure of contextual fear

conditioning there was no group effect on % time freezing ($F(2, 29) = 0.517$, $p < 0.602$, Fig. 5.3.2C.

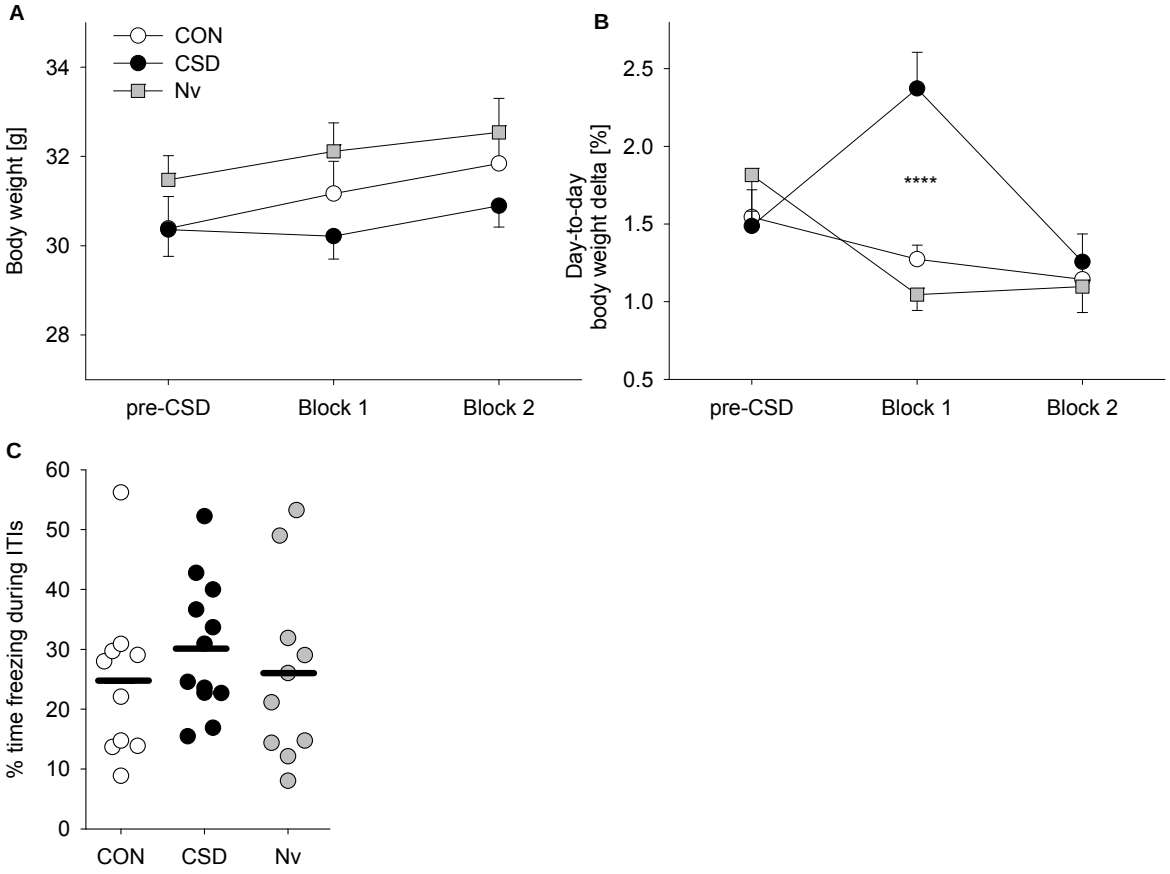

Figure 5.3.2: Effects of chronic social defeat on body weight and contextual fear conditioning, two measures of CSD efficacy. (A) Absolute body weight. (B) Percent day-to-day ΔBW. (C) Per cent time freezing during inter-trial intervals (ITIs) in the fear conditioning test. Values are mean +/- SD. **** $p < 0.0005$.

After one day of adaptation to the sleep laboratory, sleep was assessed during a 24-h baseline followed by a 4-h sleep deprivation and a 20-h recovery period. First, vigilance states during baseline were compared in CSD mice and CON mice. Both groups exhibited typical light-dark levels of Wake, REM sleep and NREM sleep, with a predominance of Wake during the dark period and a predominance of NREM sleep during the light period (Fig. 5.3.3). There was no significant effect of CSD on baseline

Wake (light period: $p = 0.72$, dark period: $p = 0.79$) or baseline NREM sleep (light period: $p = 0.75$, dark period: $p = 0.93$). For REM sleep there was no group effect during the light period ($p = 0.67$). During the dark period there was a significant main effect of group, indicating the reduction in REM sleep by CSD relative to CON mice ($F(1,13) = 4.856$, $p<0.05$ (Fig. 5.3.3). There were no significant effects of CSD on vigilance states in the second day of recording, neither during the 4-h sleep deprivation nor during recovery.

The homeostatic regulation of sleep is reflected by ECoG slow wave activity (SWA, 1-4 Hz). Under baseline conditions, SWA followed a similar time course in both groups (Fig. 5.3.4A). After 4-h sleep deprivation, CSD mice exhibited a reduced increase in SWA during the remaining 8 hours of the light period relative to CON mice: main effect of group ($F(1,13) = 5.547$, $p<0.05$). After sleep deprivation, the ECoG power differences between CSD mice and CON were not restricted to the delta frequency range (1-4 Hz) but extended to the theta frequency range (6-10 Hz, Fig. 5.3.5). Because SWA increases not only as a function of prior wakefulness but also depends on wake quality (Huber et al., 2007), CSD and CON mice were compared in terms of behaviours during sleep deprivation: there were no behavioural differences ($p \geq 0.15$ for all assessed behaviours).

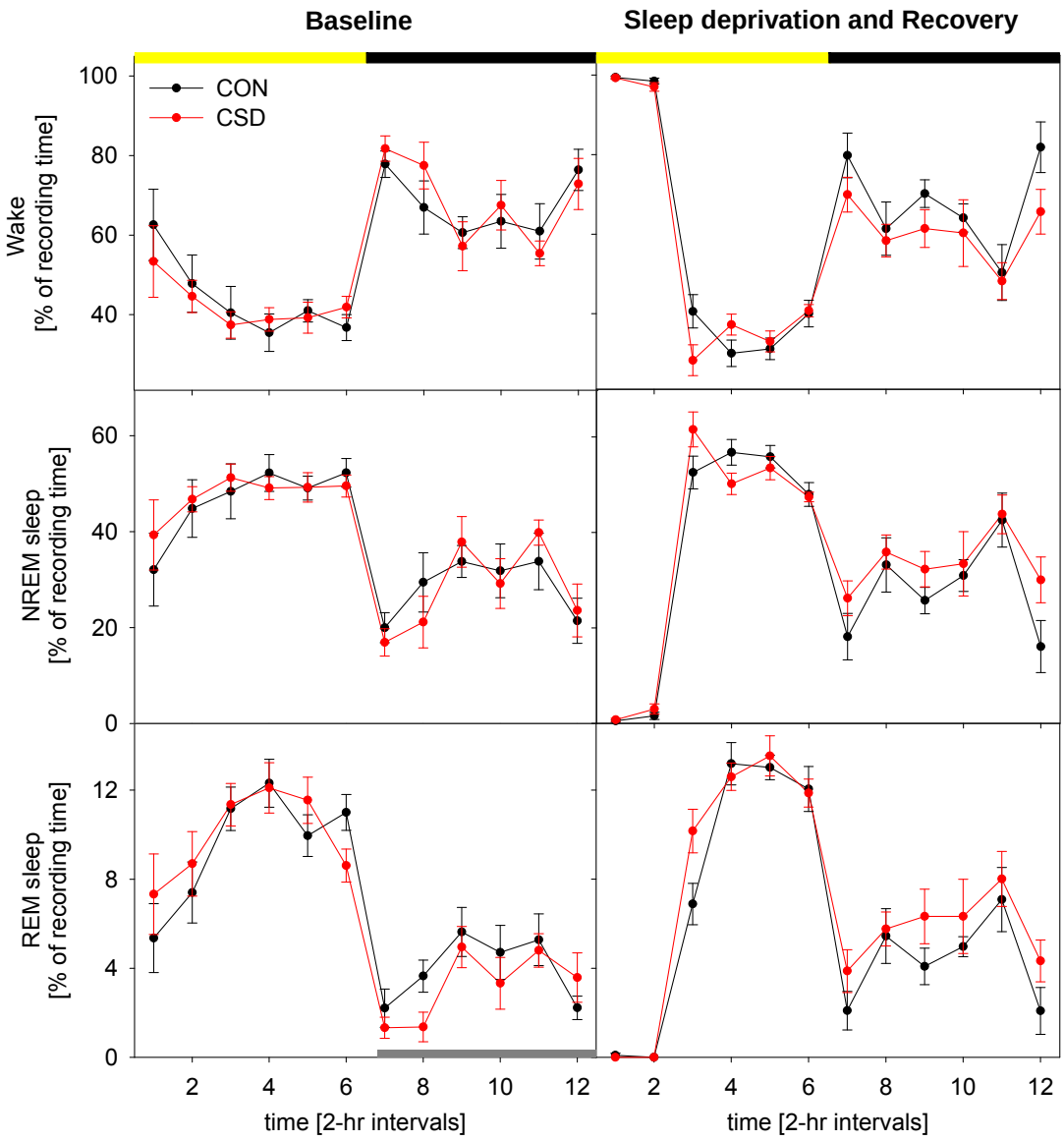

Figure 5.3.3: Vigilance states during baseline (BL), sleep deprivation (SD) and recovery (Rec). The yellow and black bars on top of the graph represent the 12-h light and dark period, respectively. Wake, NREM and REM sleep are shown in 2-h intervals for chronic social defeat (CSD) and control (CON) mice. Values are expressed as a percentage of 2 hours recording time. Intervals are shown for the BL starting at light onset, followed by 4-h SD and 20-h Rec. An ANOVA with group (CSD and CON mice) as a between-subject factor and 2-h intervals as a within-subject factor revealed a significant group effect ($p<0.05$) illustrated by the grey bar.

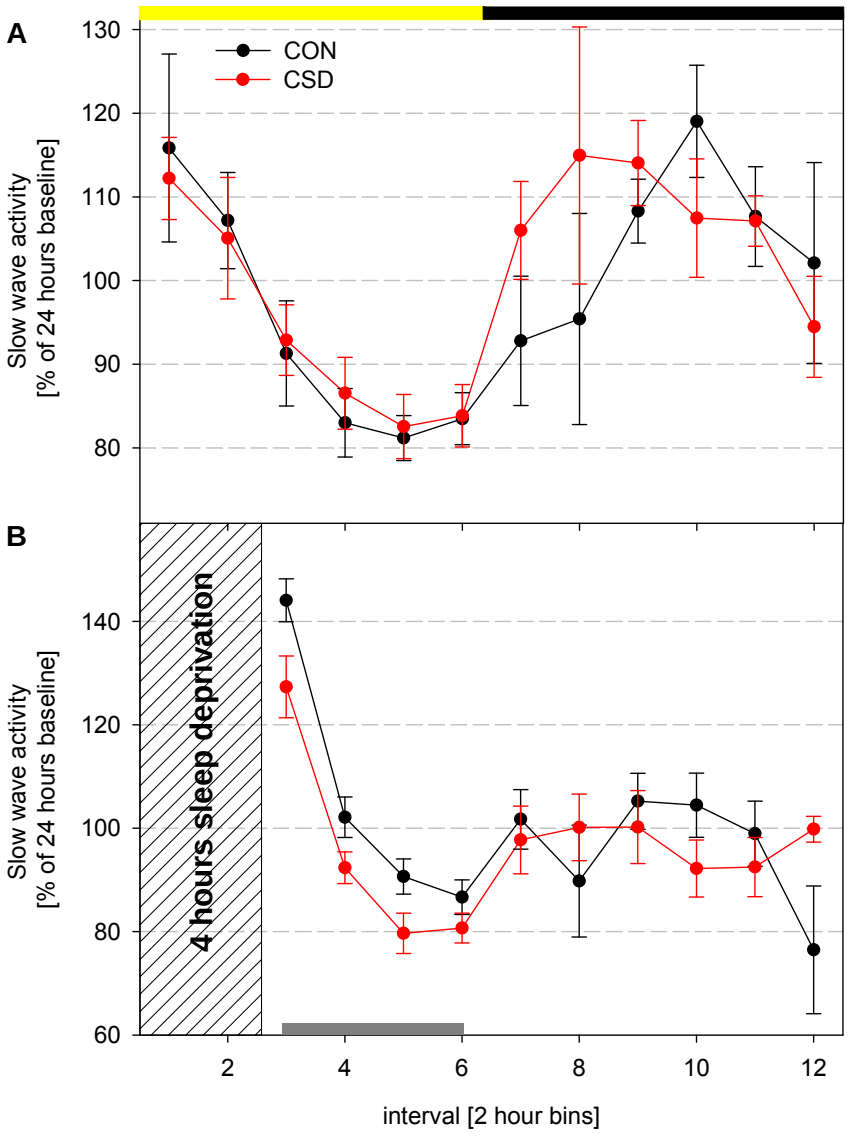

Figure 5.3.4: Slow wave activity (SWA) during baseline (BL) and recovery (Rec). The yellow and black bars on top of the graph represent the 12-h light and dark period, respectively. SWA is shown in 2-h intervals for chronic social defeat (CSD) and control (CON) mice during BL, starting at light onset (**A**) and during the 20-h Rec (**B**). Values are expressed as a percentage of the 24-h baseline. An ANOVA with group (CSD and CON) as a between factor and 2-h intervals as a within factor revealed a significant group effect ($p<0.05$) illustrated by the grey bar.

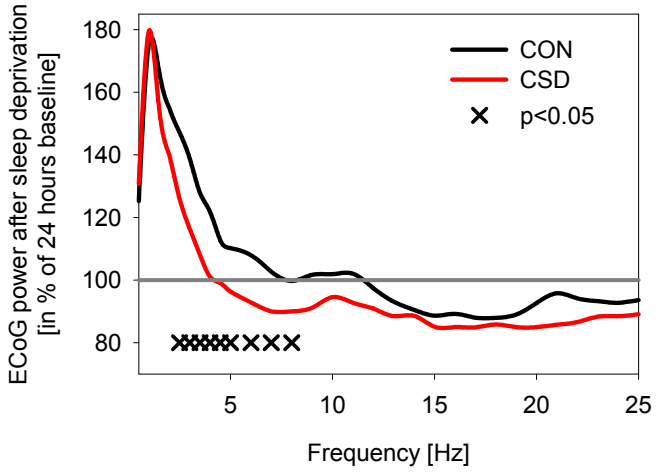

Figure 5.3.5: ECoG power spectrum differences after sleep deprivation (SD). The power spectra of the first 2-h after SD are shown for CSD and CON mice as a percentage of 24-h baseline. Crosses indicate differences between CSD mice and CON (unpaired Student's t-test, $p<0.05$).

DISCUSSION

Sleep disturbances and major depressive disorder (MDD) have been shown to be closely related (DSM-5, 2013 , ICD-10, 1994). Moreover, the S-deficiency hypothesis claims that humans suffering from depression show altered sleep homeostasis characterized by a slower accumulation of SWA (Borbely, 1987). Therefore we investigated the homeostatic regulation of sleep in a recently developed mouse model of depression (Azzinnari et al., submitted). We found no differences in the time course of SWA during a 24-h baseline. However, a 4-h sleep deprivation led to a blunted increase of SWA during the remaining light period in CSD compared to CON mice.

As an electrophysiological marker of sleep homeostasis, SWA depends on prior sleep-wake history (Borbely and Achermann, 2005) and thus changes in vigilance states may have accounted for the blunted increase in SWA after SD. However, with the exception of the reduced amount of REM sleep during the baseline dark period,

the vigilance states did not differ in CSD compared to CON mice. It was shown that SWA not only depends on quantity of wakefulness but also on its quality, i.e. the amount of prior exploratory behavior (Huber et al., 2007). Thus, behavioural differences during the SD might explain our observation. However, we did not find any behavioural differences between CSD and CON mice during the SD. Thus, neither alterations in vigilance states nor behavioural differences during SD may account for the sleep deprivation induced blunted increase in SWA in CSD mice.

The blunted increase in SWA in CSD mice was not restricted to the SWA frequency band but extended in the theta frequency range (5-8 Hz). This extension is in line with a recent study performed in rats which found that chronic stress exposure led to a flattening of sleep homeostasis which was not restricted to the delta frequency range but also affected the theta frequency range (Mrdalj et al., 2013).

In order to optimize its compatibility with the surgical implantation and maintenance of the ECoG implant, it was decided to modify the CSD procedure for the present study. These modifications, primarily the, reduction from 15 to 10 days of social defeat, might have reduced CSD efficacy. Also, exposure of CON mice to surgery could have impacted on their sleep status. To assess these effects, two CSD efficacy measures were studied, namely daily body weight delta and contextual fear conditioning (Azzinnari et al., submitted). For the former, the expected increase in daily BWΔ in CSD relative to CON mice was obtained and, furthermore, there was no difference on this measure between CON and Naive mice. In contrast to the standard CSD protocol, CSD mice did not exhibit increased contextual fear conditioning relative to CON mice. CON mice (and also Nv mice) exhibited higher average freezing than typically observed in naive controls whereas CSD mice exhibited less average freezing than typically observed. Therefore, it can be concluded that the methodology used to study sleep ECoG does not confound the effects of CSD on basal physiological processes (e.g. those controlling body weight) but does confound the measurement of behavioural reactivity to emotional stimuli (e.g. fear conditioned freezing).

With regard to vigilance states, there was a specific CSD effect in terms of a reduction in REM sleep during the baseline dark period. This finding is similar to another study that investigated the effect of chronic stress exposure on sleep. Sanford et al. found reduced REM sleep in mice after undergoing a fear-conditioning

training for 4 consecutive days (Sanford et al., 2003). Moreover, re-exposure to a tone previously paired with a shock was sufficient to reduce REM sleep after 4 to 5 days. Accordingly, re-exposure to the stressor (CD-1 mouse) by means of sensory communication due to the perforated divider might have induced a similar effect. Studies in humans suffering from major depression showed inconsistent results concerning REM sleep: both increased (Rao et al., 2002) and unchanged (Frey et al., 2012) levels of REM sleep have been reported. These studies show that REM sleep alteration is not a specific marker related to stress or depression in humans and in animal models.

The present study is not the first rodent study investigating the effects of psychosocial stress on sleep homeostasis. A prior study in rats found, in contrast to the present findings, an increase in SWA during NREM sleep after social stress (Meerlo et al., 1997). However, this study investigated the impact of a single social defeat of 1 hour whereas we investigated the effect of a chronic social defeat procedure. Thus, the contrary effects on sleep homeostasis might be related to different effects of acute or a chronic exposure. The impact of chronic stress was investigated by Mrdalj et al. by early postnatal stress exposure combined with additional chronic stress exposure during adulthood. Chronic stress exposure blunted sleep homeostasis as observed in our study (Mrdalj et al., 2013). Together the studies after chronic stress support the S-deficiency hypothesis (Borbely, 1987) proposing a reduced build up of SWA. But what might be the underlying mechanism of such an impaired homeostatic regulation?

Current understanding of the neuronal correlates of SWA might provide some insights. SWA was shown to be closely related to the synchronicity of cortical neuronal activity. More specifically, it was shown that the wakefulness-related increase in the amplitude of slow waves, as reflected by more SWA, was associated with a more synchronous firing pattern. While during subsequent sleep neuronal synchronicity was reduced (Vyazovskiy et al., 2009). Moreover, markers of synaptic plasticity were shown to follow a similar time course as SWA; they increased during wakefulness and decreased during subsequent sleep (Vyazovskiy et al., 2008). Taken together, SWA represents a marker of synchronous firing which is closely related to synaptic plasticity. Thus, a possible interpretation of the blunted build up of

SWA in CSD mice might be related to impaired synchronisation/synaptic potentiation mechanisms.

A brain region-specific analysis of effects of 15-day CSD on transcriptome expression has been conducted (Azzinnari et al., submitted). In medial prefrontal cortex (mPFC), CSD induced increased and decreased expression of 6 and 14 genes, respectively, relative to control mice. The upregulated genes include genes involved in immunological or stress responses (*Scn4b, Penk*) as well as plasticity related genes (*Arc*) or genes related to both (*Fos, Prkcd, Nptx2*). The biological roles of *Fos*- and *Pkrcd*-directed gene regulation are rather unspecific and depend on the nature of the stimulus (growth factors, cytokines, stress, neurotransmitters). They include several functions including differentiation, proliferation, apoptosis, immune response and depolarization of neurons (Karin et al., 1997, Choi et al., 2013, Zhu et al., 2013). *Nptx2* encodes a protein of the pentraxin family that is homologous to C-reactive and acute phase proteins in the immune system. It is involved in activity-dependent synaptic plasticity (Bjartmar et al., 2006, Goodman et al., 1996, Tsui et al., 1996), and induces the formation of new excitatory synapses and the regulation of AMPA receptor clustering at established synapses (O'Brien et al., 1999, O'Brien et al., 2002). *Arc* was shown to facilitate the removal of AMPA receptors from the plasma membrane (Chowdhury et al., 2006, Rial Verde et al., 2006), while *Arc* knockout results in increased surface AMPA expression (Shepherd et al., 2006). Taken together, although many among the upregulated genes of the transcriptome expression analysis are related to plasticity processes, the present results are not conclusive. While increased *Arc* levels are related to the removal of AMPA receptors and thus reduce synaptic strength, increased *Nptx2* facilitates the formation of excitatory synapses and thus increases synaptic strength.

Of the 14 down-regulated genes, 5_8S-rRNA, 7SK, Lars2 are associated with the protein synthesis machinery and five genes, Aspa, Mal, Mobp, Opalin, Gjc2, are functionally related to the biogenesis, stabilisation or maintenance of white matter. Myelin is of course fundamental to electrical signal conduction and activity-dependent nervous system plasticity. Using a computer modelling approach, it has been proposed that small changes in myelin thickness can have profound effects on neuronal network activity (Pajevic et al., 2013). Accordingly, based on an MRI study performed in humans, SWA was shown to be positively correlated with white matter

(Buchmann et al., 2011a). Thus, impaired white matter metabolism may have contributed to a reduced synchronization and may provide an explanation for a blunted increase in SWA in CSD mice. Moreover due to the down-regulated genes associated with impaired protein synthesis machinery, the late phase of long term potentiation may be another not mutually exclusive explanation for the blunted increase in SWA in CSD mice. A 4 hours sleep deprivation may not only have induced the early phase of long term potentiation which takes seconds up to a few hours but may also have required the late phase of long term potentiation. During this phase LTP requires gene transcription and new protein synthesis which might have been disturbed in CSD mice.

6 General discussion

The overall aim of the analyses presented in this thesis was to investigate the relationship between the sleep ECoG and cortical plasticity during both a period associated with high neuronal plasticity (development) and a period associated with reduced neuronal plasticity (mouse model of depression). In general, the studies support a close relationship between sleep slow wave activity (SWA) and cortical plasticity. This final section starts with a brief summary of the thesis' findings.

First, we could demonstrated for the first time that SWA in Sprague-Dawley rats follows an inverted U-shaped trajectory, similarly to what is observed in humans during a comparable developmental period [5.1]. SWA increases during pre-puberty followed by a decline during puberty. In addition, the manipulation of SWA by caffeine administration led to a developmental delay of electrophysiological, behavioral and structural maturational markers. Second, diurnal changes of SWA during the inverted U-shaped trajectory of SWA revealed inverse net changes of SWA across 24 hours. This was not found in more mature rats [5.2]. Third, in a recently developed mouse model of depression, sleep deprivation led to a blunted increase in SWA [5.3].

In the next section, I will first start with the discussion of the present results during a period of increased neuronal plasticity [5.1, 5.2]. To do so, I initially focus on a potential active contribution of sleep to synaptic plasticity based on the Synaptic Homeostasis Hypothesis (SHH), which provides a framework for the relationship between sleep and synaptic plasticity. Second, I broaden the focus and will discuss wakefulness as a limiting factor to our findings. Moreover, I will introduce two additional and closely related findings obtained from preliminary analyses performed during my PhD. Finally, I discuss SWA during a period associated with reduced synaptic plasticity [5.3] and conclude the discussion with an outlook of possible future directions based on the results of this thesis.

SWA during a period related to increased neuronal plasticity

Focus on sleep

SWA has been proposed not only to reflect synaptic strength but to actively contribute to a synaptic renormalisation process. This hypothesis is known as Synaptic Homeostasis Hypothesis (SHH) and this renormalisation process was suggested to be the function of sleep (Tononi and Cirelli, 2006). In brief, when new information is acquired during wakefulness, synapses are strengthened or newly built based on LTP-like plasticity principles. These plastic changes during wake require space, energy and cellular supplies. Therefore, they approach a saturation level at the end of a waking period, reaching maximal synaptic strength. The increased synaptic strength at the end of a waking period leads to more excitable neurons which can easily synchronize their activity. This synchronization results in high amplitude slow waves at the beginning of the next sleep phase (Vyazovskiy et al., 2009). The SHH postulates that these high amplitude slow waves do not only reflect synaptic strength but have a role to play. According to the hypothesis, slow waves trigger synaptic downscaling, a process that proportionally reduces overall synaptic strength. This is in the EEG/ECoG reflected by the exponential decrease of SWA. During this process strong and thus frequently used synapses get weakened whereas weak and thus rarely used synapses get eliminated. The end of a sleeping period is then associated with energy and space gains, which permit the acquisition of new information during the following waking period. In other words, SWA during adulthood plays an essential role in maintaining average synaptic strengths at levels that are energetically manageable for the tissue and that permit the acquisition of new memories by building new synapses.

Based on evidence that SWA plays an active role in synaptic renormalisation processes (Czarnecki et al., 2007, Lante et al., 2011), SWA may also play an active role in synaptic refinement processes during development. The first experimental evidence for an active role of SWA during development was reported by Kurth et al., who found that SWA in humans is involved in optimizing behavioral performances (Kurth et al., 2012). More recently Ringli and Huber have extended the SHH and proposed an active role of SWA also during development (Ringli and Huber, 2011).

This model proposes that the strengthening/formation and weakening/elimination are tilted during development. Specifically, the model proposes that during early development, synaptic strengthening prevails over synaptic downscaling leading in the long run to a net formation of synapses. In contrast, at older ages synaptic downscaling outweighs synaptic strengthening leading to a net elimination of synapses. In line with this model, SWA across 24 hours showed a gain of SWA during early development, followed by a loss of SWA later on [5.2]. In relation to the SHH, these results indicate that the decrease of SWA during a sleeping period does not counterbalance the increase in SWA during a waking period across development. As suggested by Ringli and Huber, SWA may contribute to the imbalance of synaptic strengthening/formation and synaptic weakening/elimination. In support for an active role of SWA in synaptic refinement processes during maturation, we provide the first longitudinal experimental evidence in the rat. The administration of caffeine during a critical maturational period resulted in long-lasting changes in SWA indicated by a delayed developmental SWA trajectory [5.1]. In this study caffeine administration induced an initial reduction in slow wave energy (SWE), a measurement quantifying the accumulation of SWA across time. Related to the active role of SWA, a reduction in the SWA may have limited synaptic weakening/elimination processes needed for proper cortical maturation which might have led to a delayed SWA trajectory thereafter. In contrast, we hypothesized that when SWA is enhanced, for example by means of a sleep deprivation (Borbely and Achermann, 2005), this would result in the opposing observation. We performed an additional experiment during which Sprague-Dawley rats underwent a single 4-h sleep deprivation during the same maturational period as when caffeine was administered (for comparison, see Fig. 5.1.2). The analysis of the SWA trajectory a few days after the sleep deprivation was performed revealed lower SWA in sleep deprived animals when compared to sham treated rats (Fig. 6.1). Thus, we can speculate that the manipulation of SWA during a critical developmental period changes cortical maturation in a predictable way. Specifically, reducing SWA by caffeine administration (see Fig. 5.1.2) resulted in a delay of cortical maturation while increasing SWA by sleep deprivation accelerated cortical maturation. Related to the SHH, this might indicate that the increased amount of SWA after sleep deprivation led to a stronger net reduction of synaptic strength resulting in a bigger loss of synapses. In contrast, when SWA was suppressed by

caffeine administration, this lowered the net reduction of synaptic strength resulting in a smaller loss of synapses. Note however that this result was obtained on N = 6 animals and one should therefore be careful with its interpretation until it is confirmed in a larger group.

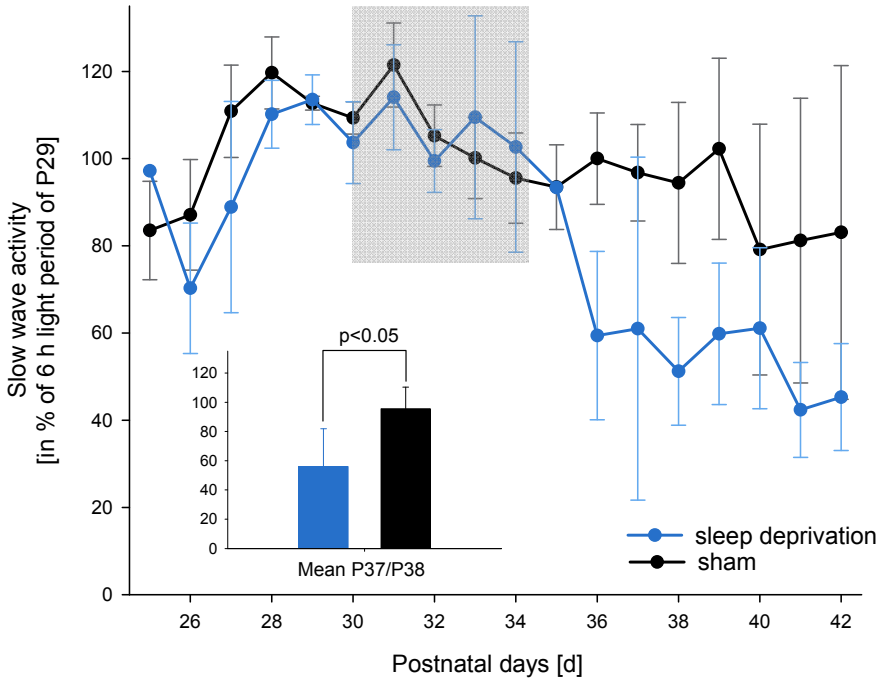

Figure 6.1: Preliminary evidence that sleep deprivation affects cortical maturation in the juvenile rat. Trajectory of sleep slow wave activity (SWA, 1- 4 Hz, averaged across the first 3 hours after light onset) between postnatal day 25 (P25) and P42 for sham (n=6) and sleep deprived (n=6) rats. The grey shaded background illustrates the period during which a 4-h sleep deprivation was performed. The inset shows an unpaired Student t-test comparison between the conditions on mean SWA at P37 and P38. Error bars indicate SEM.

Focus on wakefulness

While there is a large body of literature supporting a wake-dependent net increase in synaptic strength, there is no evidence proving a direct and thus dose-dependent relationship between SWA and synaptic weakening or elimination. Both caffeine administration and sleep deprivation affect the animal's physiology beyond

merely changing SWA. It is thus difficult to definitely confirm an active role for SWA in cortical maturation. Another aspect to consider in our experimental framework is the influence of wakefulness on cortical maturation. Indeed, experience-dependent plasticity during wakefulness was shown to have long-lasting effects when applied during a critical period. During development, the presence or absence of stimuli can lead to changes in synapse numbers: Long-term sensory deprivation reduced synapses, whereas enriched environments increased the synapse number and dendritic branching (Greenough et al., 1973, Fiala et al., 1978). For instance, classical studies performed in the visual system have shown that sensory manipulations such as monocular deprivation applied during a critical period can have long-lasting consequences (Wiesel and Hubel, 1963). These findings have been confirmed in a variety of animals and in different cortical systems (Barth et al., 1997, Issa et al., 1999, King and Moore, 1991, Doupe and Kuhl, 1999). In our experiments, both sleep deprivation and caffeine administration resulted in prolonged wakefulness. During sleep deprivation the animals were also exposed to a variety of new objects and thus experienced an enriched environment. Induced explorative behavior was shown to be closely related to the increased expression of plasticity-related genes like brain-derived neurotrophic factor (BDNF) (Huber et al., 2007). Interestingly, increased BDNF levels during a critical period were shown to accelerate cortical maturation (Huang et al., 1999). Thus, we can speculate that sleep deprivation during a critical period may have accelerated cortical maturation. Caffeine administration also led to prolonged wakefulness, however to a much lower extent. Caffeine treated rats spent the prolonged wake period in a familiar environment and no additional sensory stimulation was thus experienced. Interestingly, caffeine administration was very recently shown to delay cortical maturation during the first postnatal week in mice. The delay was due to delayed migration and insertion of GABAergic neurons into the hippocampus (Silva et al., 2013). This delay in inhibitory circuit formation led to increased neuronal network excitability. Since GABAergic innervation has not fully matured after the first postnatal week (Vincent et al., 1995) and caffeine treated rats in our study showed increased neuronal network excitability, indicated by higher SWA, it is tempting to speculate that caffeine might have influenced GABAergic signalling.

Thus far, I exposed two angles that might explain our findings. One is related to the active contribution of sleep during cortical maturation. The other considers wakefulness as an important additional factor. However, both approaches fail to provide a specific underlying cellular target mechanism. It is also probable that focusing on sleep or wakefulness only is too one-dimensional and that our findings result from a combination of processes related to both. Nevertheless, the finding of an inverted U-shaped trajectory of SWA in the rat similar to what is observed in humans during a comparable developmental period [5.1] demonstrates that rats are a good model for further investigations of these questions. In humans, SWA is not only an indicator of overall cortical maturation but was shown to provide additional topographical information. To further support the similarity in the relationship between SWA and maturation between rats and humans, I next focus on the ECoG in rats in relation to topographical changes.

SWA and topography

The thickness of cortical grey matter provides an indirect measure of synaptic maturation. Human studies have shown that changes in cortical grey matter (thickness or volume) proceed asynchronously in different brain areas. Lower-order primary areas mature early and higher-order association areas mature rather late during development (Paus, 2005, Gogtay et al., 2004). Moreover, it has been shown that peak cortical thickness follows a posterior-to-anterior trajectory (Shaw et al., 2008) (illustrated in 4.1.3) which is in line with the maturation of cortical functioning. For example, maximal visual acuity, a task predominantly performed by the primary visual cortex (occipital lobe) matures in the first years of life (Teller, 1981). During the same age span also peak cortical thickness is reached in the occipital cortex. On the other hand, executive functions, which are strongly dependent on frontal cortices (Tau and Peterson, 2010) are not fully mature until late adolescence (Spear, 2000, Luna and Sweeney, 2004). Also peak cortical thickness is reached in frontal regions rather late compared to occipital regions (Shaw et al., 2008). Interestingly, SWA was shown to be an additional marker to track topographical maturation since SWA assessed by high density EEG also follows a posterior-to-anterior maturational trajectory (Kurth et al., 2010, Kurth et al., 2012). Although cortical maturation in the

rat was mainly studied for specific brain regions or earlier during postnatal development, there is limited evidence for an asynchronous cortical maturation for different brain areas. For example, the use of an anterogradely transported tracer shows that the growth of callosal afferents follows a different time schedule in primary and secondary visual cortices (Miller and Vogt, 1984). Thus, in a subset of rats, we investigated the presence of topographical SWA differences during maturation. As shown in Fig 6.2, both derivations follow a similar inverted U-shaped trajectory in the low frequency activity (1-2 Hz). The comparison of the two derivations showed, like in humans, topographical changes during cortical maturation. The frontal location of the ECoG electrode corresponding to the primary motor cortex matures earlier whereas the parietal electrode that measured cortical activity of the primary visual cortex matures later (Paxinos and Watson, 1997). This finding is interesting since it has been shown that sensory experience is needed for structural reorganisation processes (Lendvai et al., 2000). The primary motor cortex receives sensory input relatively early during development which may lead to an earlier cortical maturation of the primary motor cortex. In contrast to the primary motor cortex, the visual cortex receives sensory input only after eye-opening which occurs not before postnatal day 14 (Johns et al., 1992). This experience-dependent reorganisation during cortical maturation may be the reason for an asynchronous cortical maturation.

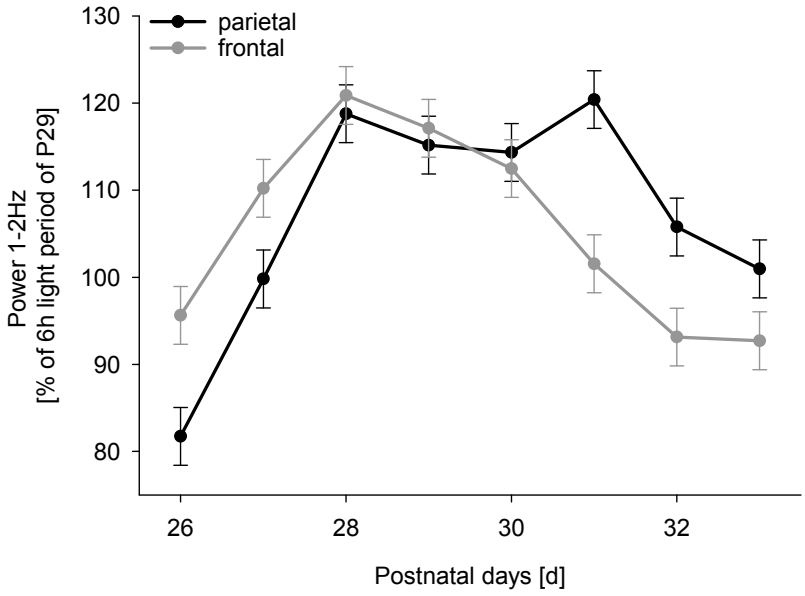

Figure 6.2: Topographical age-dependent changes of low-frequency ECoG power. Trajectory of parietal and frontal low frequency activity (ECoG power between 1 and 2 Hz, averaged over the first 3 hours after light onset) between postnatal day 26 (P26) and P33 (n=8). A two-way repeated measures ANOVA with age as a within factor (P26-P33) and location (parietal and frontal) as a between factor was significant for age and the interaction age x location ($p<0.05$).

Our results provide compelling evidence that SWA represents a useful marker of cortical maturation in the Sprague-Dawley rat. Moreover, the findings of the present thesis in the rat are very closely related to SWA changes during cortical maturation in humans [5.1] also in relation to topographical differences (Fig. 6.2). Overall, this finding strengthens the ideas that the rat is a very good model to further investigate SWA during a period of intense neuronal reorganisation. Similarly, the recently developed mouse model of depression provides a good model of depression but which relates to a reduced neuronal plasticity. The next section focuses on SWA in this disease model of reduced neuronal plasticity.

SWA during a period with decreased neuronal plasticity

In the present thesis, SWA was investigated in a recently developed mouse model of depression and the blunted increase in SWA after a 4-h sleep deprivation indicates reduced synaptic plasticity in mice that underwent chronic psychosocial stress [5.3].

Although mood disorders were initially thought to be a neurochemical disorder, there is now considerable evidence demonstrating that mood disorders are also associated with structural alterations such as significant reductions in regional central nervous system volume and cell number (neurons and glia). The majority of volumetric neuroimaging studies demonstrate reduced cortical volumes (for reviews, see Drevets, 1999, Sheline, 2000). For example, MRI studies in familial bipolar depressives and familial unipolar depressives revealed a gray matter volume reduction of 40% (for reviews see Drevets, 2000). Such abnormalities were not

limited to frontal regions but included striatal and temporal regions (for reviews, see Drevets, 1999, Mayberg et al., 2000). Based on [i] evidence for increased neuronal loss closely related to a reduction in synaptic density/connectivity in depressives and [ii] the close relationship between SWA and synaptic density/connectivity, it was expected that SWA would be altered in depressives. Several studies have reported lower SWA in patients suffering from major depressive disorders (MDD) (see for instance Hoffmann et al., 2000, Borbely et al., 1984b, Kupfer et al., 1990). These studies found reduced SWA, which is in line with the structural abnormalities observed in MRI studies. In our study, SWA levels were only reduced after prolonged wakefulness in CSD mice [5.3]. This discrepancy might be due to the different sleeping pattern between mice and humans. Humans typically stay awake for 16-h every day, enough to see a blunted increase in SWA in depressive patients. Mice, on the other hand, show a polyphasic sleeping pattern with many sleep episodes distributed across 24 hours resulting in rather short waking periods. Thus, in mice under baseline, the short waking periods may not be long enough to detect significant differences in the accumulation of SWA between CSD and CON mice. Arguing along this way implies that CSD mice also exhibit impaired synaptic potentiation at baseline but that is not detectable due to the relative brevity of the waking episodes. However, SWA differences become visible after prolonged wakefulness such as after a 4-h sleep deprivation.

On the other hand, the blunted increase in SWA might be due to different processes. Prolonged waking may involve different synaptic plasticity processes when compared to short waking periods. While short periods of wakefulness may induce the early phase of long-term potentiation (E-LTP), prolonged waking (4-h sleep deprivation) may have extended the initial E-LTP by inducing the next phase termed late-phase long-term potentiation (L-LTP). E-LTP increases synaptic strength via two mechanisms, both independent of protein synthesis: During E-LTP, synaptic strength is increased via the phosphorylation of existing AMPA receptors increasing their activity, and by inserting additional AMPA receptors into the postsynaptic membrane (Malenka and Bear, 2004). For L-LTP, gene transcription and protein synthesis are required in the postsynaptic cell. Transcriptome analyses in the mouse model of depression support this idea since genes involved in the expression of L-

LTP were found to be down-regulated in the cortex (Azzinnari et al., submitted). It may thus be that the induction of L-LTP in CSD mice is impaired.

Future Research

The present thesis draws parallels between humans and rodents in the sleep EEG/ECoG during cortical maturation as well as during adulthood in a mouse model of depression. In particular, the ECoG of Sprague-Dawley rats during cortical maturation exhibits striking similarities to humans. What takes in humans approximately 20 years to develop, we found in the rat to mature within 20 days. The present thesis thus provides an excellent basis to investigate further a potential causal involvement of SWA in cortical maturation. One way to do so could be based on manipulations recently described. For example, tones (Ngo et al., 2013) or oscillating transcranial direct current stimulation (tDCS) (Frohlich and McCormick, 2010, Ozen et al., 2010) can be used to boost SWA. Boosting SWA combined with two-photon imaging as previously done by Maret et al. (Maret et al., 2011) allows investigating the impact of SWA on structural parameters during development. With a thinned-skull preparation repeated *in vivo* two-photon imaging of a fluorescent dye allows following the growth and retractions of cortical spines. Based on this technique spine gains and losses can be longitudinally quantified after boosting SWA for example with tones. Moreover, SWA may affect synaptogenesis or pruning differently. Therefore, the role of SWA can be investigated during the period of increased synaptogenesis (<P30) and compared to the pruning period (>P30).

Additionally, emerging evidence suggests that microglia, the resident immune cells of the brain, are not only involved in immune and macrophage-like functions but also strongly influence brain development. In particular, they are thought to play a central role during the pruning period (Derecki and Kipnis, 2013, Graeber, 2010, Schafer et al., 2012, Ueno et al., 2013, Paolicelli et al., 2011). *In vivo* two-photon imaging revealed that resting microglia make brief and direct contacts with neuronal synapses whereas prolonged microglia-synapse contacts were frequently followed by the disappearance of the presynaptic bouton (Wake et al., 2009). Based on the

involvement of microglia in activity-dependent synaptic pruning, the present rat model is suited for investigating the impact of microglia on synaptic pruning (>P30) and to address the question of how sleep might facilitate or influence this process. To do so, it would be of interest to investigate the microglia-mediated synaptic pruning during sleep and wakefulness, respectively.

Increasing evidence suggests that depression is closely related to lower neurotrophic levels (for reviews, see Manji et al., 2001, Castren et al., 2007, Jiang and Salton, 2013), which might in the long run be responsible for glial and neuronal cortical losses (for reviews see Drevets, 2000). In the present mouse model of depression, we investigated SWA immediately after the induction of chronic stress. This duration might have been too short to induce a loss of neurons. Thus, it would be interesting to measure SWA as a marker of synaptic connectivity in combination with structural changes in the cortex at a later period after the mice underwent a chronic stress protocol. Moreover, the present thesis investigated the effect of chronic stress in adult mice, a developmental period associated with reduced cortical plasticity compared to the pre-pubertal and pubertal period. It would be interesting to investigate if the induction of chronic psychosocial stress during development induces similar effects on SWA or cortical plasticity when compared to the results of the present thesis. Ongoing work in our laboratory indicates that depressive onset during childhood or puberty is associated with increased SWA levels, suggesting a better connected cortical network compared to age-matched controls (Tesler and Huber, 2014, in preparation). Therefore chronic psychosocial stress during development may have opposing effects in development compared to adulthood.

7 References

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8 Curriculum Vitae

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Education

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2004 – 2007	Bachelor of Science in Biochemistry University of Zurich
2003 – 2004	Studies in Maths University of Zurich
2002 – 2003	Employee for the Swiss Federal Department of Home Affairs, Aarau, Switzerland
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9 List of Publications

PAPERS

[1] Olini, N. and Huber, R.
Sleep during all stages of human development
ESRS European Sleep Medicine Textbook
accepted for publication

[2] Olini, N., Kurth, S. and Huber, R.
The effects of caffeine on sleep and maturational markers in the rat.
PLoS ONE, 2013

[3] Olini, N. and Huber, R.
Diurnal changes in EEG sleep slow wave activity during development in rats
Journal of sleep research, 2014

[4] Olini, N., Rothfuchs, I., Azzinnari, D., Sigrist, H., Pryce, C. and Huber, R.
Sleep homeostasis in a mouse model for depressive disorders
ready to submit

10 Presentations

ORAL PRESENTATIONS

- Chronic caffeine intake delays markers of brain development in the rat
  8th Symposium of the Zurich Center for Integrative Human Physiology (ZIHP),
  Zurich, Switzerland, August 2012

- Sleep slow wave activity, cortical maturation and their manipulation by caffeine in the juvenile rat
  Swiss Society for Sleep research, Sleep medicine and Chronobiology (SSSSC),
  Zurich, Switzerland, April 2012

- Caffeine during a critical window affects sleep in the juvenile rat
  Research Colloquium of the Child Development Center,
  Children's University Hospital Zurich, Zurich, Switzerland, March 2012

- A longitudinal study of sleep EEG in juvenile rats
  2nd Symposium of the Child Development Center, Conference Center Au,
  Switzerland, August 2010

POSTER PRESENTATIONS

- Diurnal changes in EEG sleep slow wave activity during development in rats
  Olini N. and Huber R.
  3rd Forschungszentrum für das Kind (FZK)/Childrens's Research Center (CRC) Retreat,
  Schloss Au, Switzerland, October 2013

- In the young Sprague-Dawley rat sleep slow wave activity is not in balance
  Olini N., Huber R.
  Swiss Society for Sleep research, Sleep medicine and Chronobiology (SSSSC),
  Aarau, Switzerland, May 2013

- Caffeine delays maturation in the juvenile rat
  Olini N., Kurth S., Huber R.
  Swiss Society for Neuroscience (SSN),
  Geneva, Switzerland, February 2013

- Brain development is delayed after caffeine consumption in the juvenile rat
  Olini N., Kurth S., Huber R.
  2nd Forschungszentrum für das Kind (FZK)/Childrens's Research Center (CRC) Retreat,
  Schloss Au, Switzerland, October 2012

- Caffeine delays markers of maturation in juvenile rats
  Olini N., Kurth S., Huber R.
  21th Congress of the European Sleep Research Society (ESRS),
  Paris, France, September 2012

- Caffeine during the critical period delays maturation in juvenile rats
  Olini N., Kurth S., Huber R.
  Neuroscience Center Zurich (ZNZ) ,

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- Sleep slow wave activity, cortical maturation and its modification by caffeine in juvenile rats?
  Olini N., Kurth S., Huber R.
  Swiss Society for Neuroscience (SSN),
  Zurich, Switzerland, February 2012;

- Does slow wave activity reflect cortical maturation in the juvenile rat?
  Olini N., Kurth S., Huber R.
  Forschungszentrum für das Kind (FZK),
  Zurich, Switzerland, October 2011

- The maturation of sleep SWA in juvenile rats predicts their behavioural development
  Olini N., Kurth S., Huber R.
  Swiss Society for Sleep research, Sleep medicine and Chronobiology (SSSSC),
  St. Gallen, Switzerland, September 2011

- Does SWA reflect cortical maturation in the juvenile rat?
  Olini N., Kurth S., Huber R.
  Neuroscience Center Zurich (ZNZ) ,
  Zurich, Switzerland, September 2011

- Relationship between sleep slow wave activity and cortical maturation in rats
  Olini N., Kurth S., Huber R.
  Swiss society for neuroscience (SSN),
  Basel, Switzerland, March 2011

- A longitudinal study of sleep slow wave activity in juvenile rats
  Olini N., Kurth S., Huber R.
  20th Congress of the European Sleep Research Society (ESRS),
  Lisbon, Portugal, September 2010

- A longitudinal study of sleep slow wave activity in juvenile rats
  6th Symposium of the Zurich Center for Integrative Human Physiology (ZIHP),
  Zurich, Switzerland, August 2010

- A longitudinal study of sleep slow wave activity in juvenile rats
  Olini N., Kurth S., Huber R.
  Swiss society for neuroscience (SSN),
  Lausanne, Switzerland, March 2010

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